

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
9 December 2004 (09.12.2004)

PCT

(10) International Publication Number  
**WO 2004/106289 A1**

(51) International Patent Classification<sup>7</sup>: **C07C 281/06**,  
C07D 295/14, 307/52, 209/16, 317/58, 333/20, 211/56,  
207/08, 207/26, C07K 5/06, A61K 31/175, A61P 29/00,  
A61K 31/38, 31/40, 31/435

(21) International Application Number:  
PCT/DK2004/000371

(22) International Filing Date: 27 May 2004 (27.05.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
PA 2003 00810 30 May 2003 (30.05.2003) DK  
60/474,424 30 May 2003 (30.05.2003) US  
PA 2003 01365 19 September 2003 (19.09.2003) DK

(71) Applicant (for all designated States except US): **COMBIO**  
A/S [DK/DK]; Vesterbrogade 188, DK-1800 Frederiksberg  
C (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BONDEBJERG**,  
Jon [DK/DK]; Lyngbyvej 54, DK-2100 Copenhagen Ø  
(DK). **FUGLSANG**, Henrik [DK/DK]; Engelsborgvej  
22, DK-2800 Lyngby (DK). **NÆRUM**, Lars [DK/DK];  
Alrunevej 14, DK-2900 Hellerup (DK).

(74) Agent: **ALBIHNS A/S**; H.C. Andersens Boulevard 49,  
DK-1553 Copenhagen V (DK).

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GI, GM, HR, HU, ID, IL, IN, IS, JP, KE,  
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,  
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,  
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,  
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,  
ZW.

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GI,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,  
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- with amended claims
- under Rule 91.1(f), with a request for rectification

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **PROTEASE INHIBITORS**

(57) Abstract: A monoacyl semicarbazide of the general formula (I): (I), or a pharmaceutically acceptable salt or ester thereof, is capable of selectively inhibiting dipeptidyl-peptidase I (DPP-I), also known as cathepsin C. A compound of the invention is useful as an active substance for the treatment of inflammation, type 2 diabetes, asthma, severe influenza, respiratory syncytial virus infection, CD8 T cell inhibition, inflammatory bowel diseases, psoriasis, atopic dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease, periodontitis, rheumatoid arthritis, Huntington's disease, Chaga's disease, Alzheimer's disease, sepsis or for application in target cell apoptosis.

WO 2004/106289 A1

## PROTEASE INHIBITORS

### FIELD OF THE INVENTION

The present invention relates to novel protease inhibitors, more specifically to inhibitors  
5 of cysteine and/or serine proteases useful in the treatment/prevention of inflammation,  
diabetes and similar diseases in which proteases are involved, especially mast cell  
inflammatory mediated diseases. More specifically the invention relates to monoacyl  
semicarbazides capable of inhibiting dipeptidyl-peptidase I (DPP-I); also known as  
cathepsin C, an enzyme that cleaves a dipeptide from the N terminus of a polypeptide  
10 chain.

### BACKGROUND OF THE INVENTION

Dipeptidyl peptidase-I (DPP-I; EC 3.4.14.1) also known as cathepsin C is a lysosomal  
cysteine protease belonging to the papain family. The enzyme is constitutively  
15 expressed in many tissues with highest levels in lung, kidney, liver and spleen. The  
cDNAs encoding rat, human and murine DPP-I have been cloned and sequenced and  
showed that the enzyme is highly conserved. DPP-I is synthesized as an inactive  
precursor (Zymogen), and is activated by a non-autocatalytic excision of an internal  
activation peptide within the N-terminal propeptide. Once activated, DPP-I catalyzes  
20 the removal of dipeptides from the N-terminal end of polypeptide substrates with broad  
specificity. The pH optimum lies in the region of 5-7 using human DPP-I. Furthermore,  
DPP-I is oligomeric with little amino acid sequence homology compared to the  
exopeptidases cathepsin B, H, L, O and S which in addition are monomeric. Recent  
data suggests that, beside of being an important enzyme in lysosomal protein  
25 degradation, DPP-I also functions as a key enzyme in the activation of granule serine  
peptidases in cytotoxic T lymphocytes and natural killer cells (granzymes A and B),  
mast cells (chymase and tryptase), and neutrophils (cathepsin G and elastase).

Mast cells are found in many tissues, but are present in greater numbers along the  
30 epithelial linings of the body, such as the skin, respiratory tract and gastrointestinal  
tract. Mast cells are also located in the perivascular tissue surrounding small blood  
vessels. In humans, two types of mast cells have been identified. The T-type, which  
expresses only tryptase, and the MC-type, which expresses both tryptase and  
chymase. In humans, the T-type mast cells are located primarily in alveolar tissue and  
35 intestinal mucosa while the TC-type cells predominate in skin and conjunctiva. Mast  
cells can release a range of potent inflammatory mediators including cytokines,

leukotrienes, prostaglandins, histamine and proteoglycans, but among the most abundant products of mast cell activation are the serine proteases of the chymotrypsin family; tryptase and chymase. These proteases are situated in the mast cell lysosomes as fully active enzymes. The exact site of tryptase and chymase activation from  
5 zymogen precursors is not known, but the Golgi apparatus might play a role in that regard. DPP-I, which is particularly abundant in mast cells, seems to be the key enzyme responsible for activation of chymase and tryptase. Moreover, tryptase and chymase are emerging as important mediators of allergic diseases. After secretion from activated mast cells, there is evidence that these proteases are heavily involved in  
10 processes of inflammation, tissue remodelling, bronchoconstriction and mucus secretion, which have made these proteases attractive for therapeutic intervention. Mast cells seem also to play a role in angiogenesis since these cells accumulate in many angiogenesis-dependent situations. Moreover, several mast cell mediators (e.g. histamine, chymase, VEGF and bFGF) are found to be angiogenic and regulate  
15 endothelial cell proliferation and function.

Neutrophils cause considerable damage in a number of pathological conditions. When activated, neutrophils secrete destructive granular enzymes including elastase and cathepsin G and undergo oxidative bursts to release reactive oxygen intermediates.  
20 Numerous studies have been conducted on each of these activating agents in isolation. Pulmonary emphysema, cystic fibrosis and rheumatoid arthritis are just some examples of pathological conditions associated with the potent enzymes elastase and cathepsin G.

25 The strong evidence associating tryptase and chymase with a number of mast cell mediated allergic, immunological and inflammatory diseases, and the fact that cathepsin G and elastase also seem to play important roles in these types of diseases points out DPP-I as an attractive target enzyme for therapeutic intervention against the above mentioned diseases, due to its central role in activating these proteases.  
30

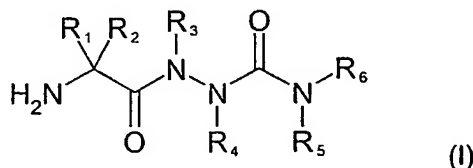
US 5,602,102 to The University of Texas discloses inhibitors of dipeptidyl peptidase I.

35

WO03/022871A2 to Probiodrug discloses inhibitors of dipeptidyl peptidase I.

## SUMMARY OF THE INVENTION

The present invention relates to compounds of the general formula (I)



wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are as defined in the detailed part of this description, or a pharmaceutically acceptable salt or ester thereof.

- 10 It is contemplated that the compounds of the invention are useful for the treatment of inflammation or type 2 diabetes, particularly for treatment of prevention of mast cell inflammatory mediated diseases such as asthma, severe influenza, respiratory syncytial virus infection, CD8 T cell inhibition, inflammatory bowel diseases, psoriasis, atopic dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease, periodontitis, rheumatoid arthritis, Huntington's disease, Chagas' disease, Alzheimer's disease or sepsis. The compounds of the present invention are especially applicable in target cell apoptosis.

15 In accordance with the present invention there is provided compounds, which are contemplated as being useful as in vitro and in vivo diagnostic tools.

It is an object of the invention to provide novel compounds having pharmacological activity as inhibitors of proteases, particularly serine and/or cysteine proteases, more particularly cysteine proteases, even more particularly cysteine proteases of the papain superfamily, yet more particularly dipeptidyl-peptidase I.

Further objects will become apparent from the following description.

## DETAILED DESCRIPTION OF THE INVENTION

30

Definitions

The term "DPP-I" or "DPPI" as used herein is intended to mean dipeptidyl-peptidase I (EC 3.4.14.1) also known as cathepsin C, cathepsin J, dipeptidyl aminopeptidase I and



dipeptidyl transferase. DPPI cleaves a dipeptide Xaa-Xbb from the N terminus of a polypeptide chain Xaa-Xbb-Xcc-[Xxx]<sub>n</sub>, except when Xaa is Arg or Lys, or when Xbb or Xcc is Pro.

- 5 The term "treatment" is defined as the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of a compound of the present invention to prevent the onset of the symptoms or the complications, or alleviating the symptoms or the complications, or eliminating the disease, condition, or disorder.

10

As used herein, alone or in combination, the term "C<sub>1-6</sub> alkyl" denotes a straight or branched, saturated hydrocarbon chain having from one to six carbon atoms. C<sub>1-6</sub> alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, n-pentyl, iso-pentyl, 2-methylbutyl, 3-methylbutyl, n-hexyl, iso-hexyl, 4-methylpentyl, neopentyl, 2,2-dimethylpropyl and the like.

15

As used herein, alone or in combination, the term "C<sub>2-6</sub> alkenyl" denotes a straight or branched, unsaturated hydrocarbon chain having from two to six carbon atoms and at least one double bond. C<sub>2-6</sub> alkenyl groups include, but are not limited to, vinyl, 1-propenyl, allyl, iso-propenyl, n-butenyl, n-pentenyl, n-hexenyl and the like.

20

As used herein, alone or in combination, the term "C<sub>2-6</sub> alkynyl" denotes a straight or branched, unsaturated hydrocarbon chain having from two to six carbon atoms and at least one triple bond. C<sub>2-6</sub> alkynyl groups include, but are not limited to, -C≡CH, -C≡CCH<sub>3</sub>, -CH<sub>2</sub>C≡CH, -CH<sub>2</sub>-CH<sub>2</sub>C≡CH, -CH(CH<sub>3</sub>)C≡CH and the like.

25

The term "C<sub>1-6</sub> alkoxy" in the present context designates a group O-C<sub>1-6</sub> alkyl used alone or in combination, wherein C<sub>1-6</sub> alkyl is as defined above. Examples of straight alkoxy groups are methoxy, ethoxy, propoxy, butoxy, pentoxy and hexoxy. Examples of branched alkoxy are iso-propoxy, sec-butoxy, tert-butoxy, iso-pentoxy and iso-hexoxy. Examples of cyclic alkoxy are cyclopropyloxy, cyclobutyloxy, cyclopentyloxy and cyclohexyloxy.

30

The term "C<sub>1-6</sub> alkylthio" in the present context designates a group -S-C<sub>1-6</sub> alkyl wherein C<sub>1-6</sub> alkyl is as defined above. Representative examples include, but are not limited to, methylthio, ethylthio, n-propylthio, isopropylthio, butylthio, isobutylthio, sec-butylthio,

35

tert-butylthio, n-pentylthio, isopentylthio, neopentylthio, tert-pentylthio, n-hexylthio, isohexylthio and the like.

5 The term "C<sub>1-6</sub> alkylcarbonyl" in the present context designates a group -(CO)-C<sub>1-6</sub> alkyl wherein C<sub>1-6</sub> alkyl is as defined above. Representative examples include, but are not limited to, methylcarbonyl, ethylcarbonyl, n-propylcarbonyl, isopropylcarbonyl, butylcarbonyl, isobutylcarbonyl, sec-butylcarbonyl, tert-butylcarbonyl, n-pentylcarbonyl, isopentylcarbonyl, neopentylcarbonyl, tert-pentylcarbonyl, n-hexylcarbonyl, isohexylcarbonyl and the like.

10

The term "C<sub>1-6</sub> N-alkylamide" in the present context designates a group -(CO)NH-C<sub>1-6</sub> alkyl, wherein C<sub>1-6</sub> alkyl is as defined above. Representative examples include, but are not limited to, N-methylamide, N-ethylamide, N-propylamide, N-butylamide, N-pentylamide and N-hexylamide.

15

The term "dialkylamino C<sub>1-6</sub> alkyl" as used herein designates a group di-C<sub>1-4</sub>alkyl-N-C<sub>1-6</sub>alkyl, wherein C<sub>1-6</sub> alkyl is as defined above. Representative examples include, but are not limited to, dimethylaminomethyl.

20

The term "C<sub>3-10</sub> cycloalkyl" as used herein denotes a radical of one or more saturated mono-, bi-, tri- or spirocyclic hydrocarbon having from three to ten carbon atoms. Examples include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, bicyclo[3.2.1]octyl, spiro[4.5]decyl, norpinyl, norbornyl, norcaryl, adamantyl and the like.

25

The term "C<sub>5-10</sub> cycloalkenyl" as used herein denotes a radical of one or more saturated cyclic hydrocarbon having from five to ten carbon atoms and at least one double bond. Examples include, but are not limited to, cyclopentenyl and cyclohexenyl and the like.

30

The term "C<sub>3-7</sub> heterocycloalkyl" as used herein denotes a radical of a totally saturated heterocycle like a cyclic hydrocarbon containing one or more heteroatoms selected from nitrogen, oxygen and sulphur independently in the cycle. Examples of heterocycles include, but are not limited to, pyrrolidine (1-pyrrolidine, 2-pyrrolidine, 3-pyrrolidine, 4-pyrrolidine, 5-pyrrolidine), pyrazolidine (1-pyrazolidine, 2-pyrazolidine, 3-pyrazolidine, 4-pyrazolidine, 5-pyrazolidine), imidazolidine (1-imidazolidine, 2-imidazolidine, 3-imidazolidine, 4-imidazolidine, 5-imidazolidine), thiazolidine (2-

35

thiazolidine, 3-thiazolidine, 4-thiazolidine, 5-thiazolidine), piperidine (1-piperidine, 2-piperidine, 3-piperidine, 4-piperidine, 5-piperidine, 6-piperidine), piperazine (1-piperazine, 2-piperazine, 3-piperazine, 4-piperazine, 5-piperazine, 6-piperazine), morpholine (2-morpholine, 3-morpholine, 4-morpholine, 5-morpholine, 6-morpholine),  
5 thiomorpholine (2-thiomorpholine, 3-thiomorpholine, 4-thiomorpholine, 5-thiomorpholine, 6-thiomorpholine), 1,2-oxathiolane (3-(1,2-oxathiolane), 4-(1,2-oxathiolane), 5-(1,2-oxathiolane)), 1,3-dioxolane (2-(1,3-dioxolane), 3-(1,3-dioxolane), 4-(1,3-dioxolane)), tetrahydropyran (2-tetrahydropyran, 3-tetrahydropyran, 4-tetrahydropyran, 5-tetrahydropyran, 6-tetrahydropyran), hexahydropyridazine, (1-  
10 (hexahydropyridazine), 2-(hexahydropyridazine), 3-(hexahydropyridazine), 4-(hexahydropyridazine), 5-(hexahydropyridazine), 6-(hexahydropyridazine)).

The term "aryl" as used herein is intended to include carbocyclic aromatic ring systems. Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic  
15 systems enumerated below.

The term "heteroaryl" as used herein includes heterocyclic aromatic ring systems containing one or more heteroatoms selected from nitrogen, oxygen and sulphur such as furyl, thienyl, pyrrolyl, and is also intended to include the partially hydrogenated  
20 derivatives of the heterocyclic systems enumerated below.

Examples of "aryl" and "heteroaryl" includes, but are not limited to, phenyl, biphenyl, indenyl, naphthyl (1-naphthyl, 2-naphthyl), N-hydroxytetrazolyl, N-hydroxytriazolyl, N-hydroxyimidazolyl, anthracenyl (1-anthracenyl, 2-anthracenyl, 3-anthracenyl), phenanthrenyl, fluorenyl, pentalenyl, azulenyl, biphenylenyl, thiophenyl (1-thienyl, 2-  
25 thienyl), furyl (1-furyl, 2-furyl), furanyl, thiophenyl, isoxazolyl, isothiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, pyranyl, pyridazinyl, pyrazinyl, 1,2,3-triazinyl, 1,2,4-triazinyl, 1,3,5-triazinyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, tetrazolyl, thiadiazinyl, indolyl, isoindolyl, benzofuranyl, benzothiophenyl (thianaphthenyl), indolyl,  
30 oxadiazolyl, isoxazolyl, quinazolinyl, fluorenyl, xanthenyl, isoindanyl, benzhydryl, acridinyl, benzisoxazolyl, purinyl, quinazolinyl, quinoliziny, quinoliny, isoquinoliny, quinoxaliny, naphthyridiny, pteridinyl, azepiny, diazepiny, pyrrolyl (2-pyrrolyl), pyrazolyl (3-pyrazolyl), imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl, 1,2,3-triazol-4-yl, 1,2,4-triazol-  
35 3-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), thiazolyl (2-thiazolyl, 4-thiazolyl, 5-thiazolyl), pyridyl (2-pyridyl, 3-pyridyl, 4-pyridyl), pyrimidinyl (2-pyrimidinyl, 4-

pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl), pyrazinyl, pyridazinyl (3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), isoquinolyl (1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), quinolyl (2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-quinolyl, 7-quinolyl, 8-quinolyl), benzo[b]furanyl (2-  
5 benzo[b]furanyl, 3-benzo[b]furanyl, 4-benzo[b]furanyl, 5-benzo[b]furanyl, 6-benzo[b]furanyl, 7-benzo[b]furanyl), 2,3-dihydro-benzo[b]furanyl (2-(2,3-dihydro-benzo[b]furanyl), 3-(2,3-dihydro-benzo[b]furanyl), 4-(2,3-dihydro-benzo[b]furanyl), 5-(2,3-dihydro-benzo[b]furanyl), 6-(2,3-dihydro-benzo[b]furanyl), 7-(2,3-dihydro-benzo[b]furanyl)), benzo[b]thiophenyl (2-benzo[b]thiophenyl, 3-benzo[b]thiophenyl, 4-  
10 benzo[b]thiophenyl, 5-benzo[b]thiophenyl, 6-benzo[b]thiophenyl, 7-benzo[b]thiophenyl), 2,3-dihydro-benzo[b]thiophenyl (2-(2,3-dihydro-benzo[b]thiophenyl), 3-(2,3-dihydro-benzo[b]thiophenyl), 4-(2,3-dihydro-benzo[b]thiophenyl), 5-(2,3-dihydro-benzo[b]thiophenyl), 6-(2,3-dihydro-benzo[b]thiophenyl), 7-(2,3-dihydro-benzo[b]thiophenyl)), indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl,  
15 7-indolyl), indazolyl (1-indazolyl, 2-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl, (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl,  
20 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl). Non-limiting examples of partially hydrogenated derivatives are 1,2,3,4-tetrahydronaphthyl, 1,4-dihydronaphthyl, pyrrolinyl, pyrazolinyl, indolinyl, oxazolidinyl, oxazolinyl, oxazepinyl and the like.

25 The term "aryl-C<sub>1-5</sub>alkyl" as used herein refers to a C<sub>1-5</sub>-alkyl group as defined above having one, two, three, four or five carbon atoms attached through an aryl group as defined above.

The term "heteroaryl-C<sub>1-5</sub>alkyl" as used herein refers to a C<sub>1-5</sub>-alkyl is as defined above  
30 having one, two, three, four or five carbon atoms attached through a heteroaryl group as defined above.

The term "C<sub>1-6</sub>-alkylaryl" as used herein refers to an aryl group as defined above attached through a C<sub>1-6</sub>alkyl group as defined above having one, two, three, four, five  
35 or six carbon atoms.

The term "C<sub>1-6</sub>-alkylheteroaryl" as used herein refers to a heteroaryl group as defined above attached through a C<sub>1-6</sub>alkyl group as defined above having one, two, three, four, five or six carbon atoms.

- 5 The term "aroyl" as used herein represents a group -(CO)-aryl wherein aryl is as defined above.

The term "C<sub>3-7</sub>cycloalkyl-C<sub>1-5</sub>alkyl" as used herein refers to a cycloalkyl group as defined above attached through an alkyl group as defined above having the indicated  
10 number of carbon atoms.

The term "C<sub>3-7</sub>heterocycloalkyl-C<sub>1-5</sub>alkyl" as used herein refers to a heterocycloalkyl group as defined above attached through an alkyl group as defined above having the indicated number of carbon atoms.

15

"Halogen" designates an atom selected from the group consisting of F, Cl, Br and I.

The terms "unsubstituted" or "substituted" as used herein means that the groups in question are optionally unsubstituted or substituted with one, two or three substituents  
20 independently of each other selected from C<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkylthio, C<sub>1-6</sub>alkylcarbonyl, C<sub>1-6</sub>-N-alkylamide, C<sub>1-6</sub>alkoxy, dialkylamino-C<sub>1-6</sub>alkyl, amide, hydroxy, carboxy, amino, halogen, trifluoromethyl, trifluoromethoxy, trifluoromethylthio and cyano. When the groups in question are substituted with more than one substituent the substituents may be the same or different.

25

The terms "amino acid", "amino acid residue", "natural amino acid" and "natural amino acid residue" as used herein all refer to the D- or L- isomers of the 20 standard amino acid residues: alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His),  
30 isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val).

The terms "unnatural amino acid" and "non-natural amino acid residue" as used herein  
35 refer to non-standard or modified or unnatural amino acid residues. Examples of non-standard amino acid residues are 4-hydroxyproline, 6-N-methyl lysine, 2-

aminoisobutyric acid, isovaline, and alpha-methyl serine. Examples of unnatural amino acid residues are pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, and 3,3-dimethylproline.

- 5 Certain of the above defined terms may occur more than once in the structural formulae, and upon such occurrence each term shall be defined independently of the other.

- 10 As used herein, the phrase "a functional group which can be converted to hydrogen in vivo" is intended to include any group, which upon administering the present compounds to the subjects in need thereof can be converted to hydrogen enzymatically or by the acidic environment in the stomach. Non-limiting examples of such groups are acyl, carbamoyl, monoalkylated carbamoyl, dialkylated carbamoyl, alkoxycarbonyl including C<sub>1-6</sub>-alkoxycarbonyl, alkoxyalkyl groups including C<sub>1-6</sub>-alkoxy- C<sub>1-6</sub>-alkyl and 15 the like such as C<sub>1-6</sub>-alkylcarbonyl, aroyl, C<sub>1-6</sub>-alkylcarbamoyl (C<sub>1-6</sub>-N-alkylamide) and di-C<sub>1-6</sub> alkyl-alkylcarbamoyl.

- As used herein, the phrase "diseases and disorders related to dipeptidyl-peptidase I" is intended to include any disease or disorder in which an effect, preferably an inhibiting 20 effect, on the dipeptidyl-peptidase I enzyme is beneficial.

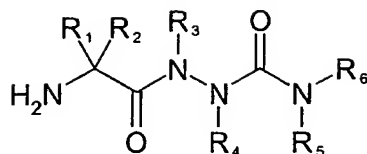
The term "IC<sub>50</sub>" as used herein denotes the concentration required for 50% inhibition of DPP-I in a binding assay described herein (DPP-I assay).

- 25 Abbreviations and symbols commonly used in the peptide and chemical arts are used herein to describe the compounds of the present invention. In general, the amino acid abbreviations follow the IUPAC-IUB Joint Commission on Biochemical Nomenclature as described in Eur. J. Biochem., 158, 9 (1984).

- 30 Certain radical groups are abbreviated herein. t-Bu refers to the tertiary butyl radical, Boc refers to the t-butyloxycarbonyl radical, Fmoc refers to the fluorenylmethoxycarbonyl radical, Ph refers to the phenyl radical, Cbz refers to the benzyloxycarbonyl radical.

- 35 The compounds

The present invention relates to a compound of formula (I)



(I)

or a pharmaceutically acceptable salt or ester thereof,

5 wherein

$R_1$  and  $R_2$  independently of each other are hydrogen,  $C_{1-6}$ alkyl,  $C_{2-6}$ alkenyl,  $C_{2-6}$ alkynyl, an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group, an unsubstituted or substituted  $C_{5-10}$ cycloalkenyl group, an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group, an unsubstituted or substituted  $C_{1-6}$ alkylaryl group but not benzyl when  $R_6$  are phenyl, an  
 10 unsubstituted or substituted  $C_{1-6}$ alkylheteroaryl group, an unsubstituted or substituted aryl group, an unsubstituted or substituted heteroaryl group, an unsubstituted or substituted aryl- $C_{1-5}$ alkyl group, an unsubstituted or substituted heteroaryl- $C_{1-5}$ alkyl group, an unsubstituted or substituted  $C_{3-7}$ cycloalkyl- $C_{1-5}$ alkyl group, an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl- $C_{1-5}$ alkyl group, or  $R_1$  and  $R_2$  together form an  
 15 unsubstituted or substituted  $C_{3-10}$ cycloalkyl group or an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group;

$R_3$  is hydrogen,  $C_{1-6}$ alkyl, or  $R_3$  and  $R_1$  together form an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group or an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group, or  $R_3$  and  $R_2$  together form an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group or an  
 20 unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group;

$R_4$  and  $R_5$  independently of each other are hydrogen or  $C_{1-6}$ alkyl;

$R_6$  is  $C_{1-6}$ alkyl,  $-\text{CH}(R_7)\text{CONH}(R_8)$ ,  $C_{2-6}$ alkenyl,  $C_{2-6}$ alkynyl, an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group, an unsubstituted or substituted  $C_{5-10}$ cycloalkenyl group, an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group, an unsubstituted or  
 25 substituted  $C_{3-7}$ cycloalkyl- $C_{1-5}$ alkyl group, an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl- $C_{1-5}$ alkyl group, an unsubstituted or substituted  $C_{1-6}$ alkylaryl group, an unsubstituted or substituted  $C_{1-6}$ alkyl-heteroaryl group, an unsubstituted or substituted aryl group with the proviso that aryl is not substituted with a nitro group, an unsubstituted or substituted heteroaryl group, an unsubstituted or substituted aryl- $C_{1-5}$ alkyl group, an unsubstituted or substituted heteroaryl- $C_{1-5}$ alkyl group;

$R_7$  is hydrogen, an unsubstituted or substituted  $C_{1-6}$ alkyl group, an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group, an unsubstituted or substituted  $C_{1-6}$ alkylaryl group, an

unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group or a side chain of a natural or non-natural amino acid residue;

R<sub>8</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group, an  
5 unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group, a side chain of a natural or non-natural amino acid residue or a group -CH(R<sub>7</sub>)CONH(R<sub>9</sub>);

R<sub>9</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or  
10 an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group. wherein a substituted group is substituted with one, two or three substituents independently selected from the group consisting of C<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkylthio, C<sub>1-6</sub>alkylcarbonyl, C<sub>1-6</sub>-N-alkylamide, C<sub>1-6</sub>alkoxy, dialkylamino-C<sub>1-6</sub>alkyl, amide, hydroxy, carboxy, amino, halogen, trifluoromethyl, trifluoromethoxy, trifluoromethylthio and cyano.

15 R<sub>1</sub> and R<sub>2</sub> may, independently of each other, be hydrogen, C<sub>1-6</sub>alkyl, an unsubstituted or substituted phenyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group; or R<sub>1</sub> and R<sub>2</sub> may, independently of each other, be methyl, ethyl, propyl, butyl, iso-butyl, benzyl, 1-naphthylmethyl, 2-naphthylmethyl or (4-pyridyl)methyl.

20

In a specific embodiment R<sub>1</sub> is C<sub>1-6</sub>alkyl and R<sub>2</sub> is H.

In other embodiments, R<sub>1</sub> and R<sub>2</sub> together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl group such  
25 as an unsubstituted or substituted cyclohexyl group.

R<sub>3</sub> may be hydrogen or methyl.

R<sub>1</sub> and R<sub>3</sub> may together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an  
30 unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl group.

Also, R<sub>2</sub> and R<sub>3</sub> may together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl group.

35 R<sub>4</sub> and/or R<sub>5</sub> may be hydrogen or methyl.



In an interesting embodiment, at least one of R<sub>3</sub> and R<sub>4</sub> and R<sub>5</sub> is hydrogen, and in particular all of R<sub>3</sub> and R<sub>4</sub> and R<sub>5</sub> is hydrogen.

- R<sub>6</sub> may be -CH(R<sub>7</sub>)CONH(R<sub>8</sub>), C<sub>1-6</sub>alkyl, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group. Thus, R<sub>6</sub> may be an unsubstituted or substituted benzyl group.

In another embodiment R<sub>6</sub> may be the group -CH(R<sub>7</sub>)CONH(R<sub>8</sub>).

- In particular R<sub>6</sub> may be selected from 2S-3-phenylpropan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-cyclohexylpropan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-butan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-2-phenylethan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide, 2S-N-[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-3-(p-chlorophenyl)propan-2-yl-amide, 2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide, 2S-N-[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide, 2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(p-hydroxyphenyl)propan-2-yl-amide, 2S-N-[2S-4-methylpentan-2-yl-amide]-3, (benzo[b]thiophen-3-yl)propan-2-yl-amide, 2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(p-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide, 2-phenylethyl, 2-(p-hydroxyphenyl)ethyl, benzyl, o-chlorobenzyl, 2-furfuryl, 2-(3-indolyl)ethyl, 1-naphthylmethyl, 4-piperonyl, p-chlorobenzyl, 2-(thiophen-2-yl)ethyl, m-fluorobenzyl, o-fluorobenzyl, p-fluorobenzyl, o-trifluoromethylbenzyl, m-chlorobenzyl, o-methoxybenzyl, o-methylbenzyl, alpha-methylbenzyl, o-(o-hydroxymethylphenylthio)benzyl, o-bromobenzyl, 2-chloro-6-fluorobenzyl, o-ethoxybenzyl, [1,1'-biphenyl-4-yl]methyl, [1,1'-biphenyl-2-yl]methyl, p-[thiophen-2-yl]benzyl, o-[N-morpholinyl]benzyl, o-[N-piperidinyl]benzyl and o-methylthiobenzyl.

In an interesting embodiment  $R_6$  is an unsubstituted or substituted [1,1'-biphenyl-2-yl]methyl group or an unsubstituted or substituted [1,1'-biphenyl-4-yl]methyl group.

5  $R_7$  may be hydrogen, an unsubstituted or substituted  $C_{1-6}$ alkyl group, an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group, an unsubstituted or substituted  $C_{1-6}$ alkylaryl group, an unsubstituted or substituted  $C_{1-6}$ alkylheteroaryl group. Thus,  $R_7$  may be 2-phenylethyl.

10  $R_8$  may be hydrogen,  $-\text{CH}(R_7)\text{CONH}(R_9)$  or an unsubstituted or substituted  $C_{1-6}$ alkyl group.

15 Thus, when  $R_8$  is  $-\text{CH}(R_7)\text{CONH}(R_9)$ ,  $R_7$  may be an unsubstituted or substituted  $C_{1-6}$ alkylaryl group or an unsubstituted or substituted  $C_{1-6}$ alkyl-heteroaryl group. In an interesting embodiment  $R_7$  is (indol-3-yl)methyl or (m-fluorophenyl)methyl.

$R_9$  may be hydrogen or an unsubstituted or substituted  $C_{1-6}$ alkyl group. In particular,  $R_9$  is hydrogen.

20 As shown in the examples herein a representative compound of the present invention is a selective inhibitor of DPP-I, especially the compound of Example 11 has been shown to selectively inhibit DPP-I, i.e. it does not inhibit Catepsin B, G, H, L, DPP-IV, neutrophil elastase or tryptase.

Preferred compounds of the invention are:

25 1-(2S-2-Aminopropionyl)-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
1-(aminoacetyl)-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
1-(2S-2-aminopropionyl)-1-methyl-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
1-(1-aminocyclohexane-1-carbonyl)-4-[2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-cyclohexylpropan-2-yl-amide]semicarbazide;  
30 1-(1-aminocyclohexane-1-carbonyl)-4-[2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;  
1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;  
1-(2S-2-aminopropanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;  
35

- 1-(2S-3-naphthyl-2-propanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide]semicarbazide;
- 1-[2S-2-amino-3-(4-pyridyl)propanoyl]-4-[2S-N-(2S-3-(indol-3-yl)propan-2-yl-amide)-butan-2-yl-amide]semicarbazide;
- 5 1-[2S-2-amino-3-(4-pyridyl)propanoyl]-4-[2S-N-(2S-3-(indol-3-yl)propan-2-yl-amide)-2-phenylethan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide]semicarbazide;
- 10 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminopentanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;
- 15 1-(2S-2-amino-4-phenylbutanoyl)-4-[2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;
- 1-(2S-2-aminopentanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(*p*-hydroxyphenyl)propan-2-yl-amide]semicarbazide;
- 20 1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-4-methylpentan-2-yl-amide]-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 25 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*p*-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 30 1-(2S-2-Aminopentanoyl)-4-[2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide]semicarbazide;
- 35 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;

- 1-(2S-2-aminopropionyl)-4-(2-phenylethyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(2-phenylethyl)-4-methylsemicarbazide;  
1-(2S-2-aminopropionyl)-4-[2-(*p*-hydroxyphenyl)ethyl]semicarbazide;  
1-(2S-2-aminopropionyl)-4-benzylsemicarbazide;  
5 1-(2S-2-aminopropionyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(2-furfuryl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-[2-(3-indolyl)ethyl]semicarbazide;  
1-(2S-2-aminopropionyl)-4-(1-naphthylmethyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-piperonylsemicarbazide;  
10 1-(2S-2-aminopropionyl)-4-(*p*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-[2-(thiophen-2-yl)ethyl]semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*m*-fluorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*o*-fluorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*p*-fluorobenzyl)semicarbazide;  
15 1-(2S-2-aminopropionyl)-4-(*o*-trifluoromethylbenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*m*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*o*-methoxybenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*o*-methylbenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-( $\alpha$ -methylbenzyl)semicarbazide;  
20 1-(2S-2-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminovaleryl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminohexanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminoisocaproyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(1-aminocyclohexane-1-carbonyl)-4-(*o*-chlorobenzyl)semicarbazide;  
25 1-(2S-2-amino-3-phenyl-propionyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1,1-(2S-1,4-*cyclo*-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1,1-(2*R*-1,4-*cyclo*-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-[2S-*N*-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;  
30 1-(2S-2-Aminobutanoyl)-4-[*o*-(*o*-hydroxymethylphenylthio)benzyl]semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(*o*-bromobenzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(2-chloro-6-fluorobenzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(*o*-ethoxybenzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-([1,1'-biphenyl-4-yl]methyl)semicarbazide;  
35 1-(2S-2-Aminobutanoyl)-4-([1,1'-biphenyl-2-yl]methyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(*p*-[thiophen-2-yl]benzyl)semicarbazide;

- 1-(2S-2-Aminobutanoyl)-4-(*o*-[*N*-morpholinyl]benzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(1-naphthylmethyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(*o*-[*N*-piperidinyl]benzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(*o*-methylthiobenzyl)semicarbazide;  
5 1-(2*R*-2-aminopropionyl)-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
1-(2*R*-2-aminopropionyl)-1-methyl-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
1-(2*R*-2-aminobutanoyl)-4-[2S-*N*-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;  
1-(2*R*-2-aminopropanoyl)-4-[2S-*N*-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;  
10 1-(2*R*-3-naphthyl-2-propanoyl)-4-[2S-*N*-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide]semicarbazide;  
1-[2*R*-2-amino-3-(4-pyridyl)propanoyl]-4-[2S-*N*-(2S-3-(indol-3-yl)propan-2-yl-amide)-butan-2-yl-amide]semicarbazide;  
15 1-[2*R*-2-amino-3-(4-pyridyl)propanoyl]-4-[2S-*N*-(2S-3-(indol-3-yl)propan-2-yl-amide)-2-phenylethan-2-yl-amide]semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-[2S-*N*-[2S-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-[2S-*N*-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide]semicarbazide;  
20 1-(2*R*-2-Aminobutanoyl)-4-[2S-*N*-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;  
1-(2*R*-2-Aminopentanoyl)-4-[2S-*N*-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;  
25 1-(2*R*-2-amino-4-phenylbutanoyl)-4-[2S-*N*-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;  
1-(2*R*-2-aminopentanoyl)-4-[2S-*N*-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;  
1-(2*R*-2-aminobutanoyl)-4-[2S-*N*-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(*p*-hydroxyphenyl)propan-2-yl-amide]semicarbazide;  
30 1-(2*R*-2-aminobutanoyl)-4-[2S-*N*-[2S-4-methylpentan-2-yl-amide]-3-(benzo[*b*]thiophen-3-yl)propan-2-yl-amide]semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-[2S-*N*-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;  
35 1-(2*R*-2-Aminobutanoyl)-4-[2S-*N*-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;

- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(benzo[*b*]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*p*-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 5 1-(2*R*-2-Aminopentanoyl)-4-[2*S*-*N*-[2*S*-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;
- 10 1-(2*R*-2-aminopropionyl)-4-(2-phenylethyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(2-phenylethyl)-4-methylsemicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-[2-(*p*-hydroxyphenyl)ethyl]semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-benzylsemicarbazide;
- 15 1-(2*R*-2-aminopropionyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(2-furfuryl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-[2-(3-indolyl)ethyl]semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(1-naphthylmethyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-piperonylsemicarbazide;
- 20 1-(2*R*-2-aminopropionyl)-4-(*p*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-[2-(thiophen-2-yl)ethyl]semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*m*-fluorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*o*-fluorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*p*-fluorobenzyl)semicarbazide;
- 25 1-(2*R*-2-aminopropionyl)-4-(*o*-trifluoromethylbenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*m*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*o*-methoxybenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*o*-methylbenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(alpha-methylbenzyl)semicarbazide;
- 30 1-(2*R*-2-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminovaleryl)- 4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminohexanoyl)- 4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminoisocaproyl)- 4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-amino-3-phenyl-propionyl)- 4-(*o*-chlorobenzyl)semicarbazide;
- 35 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;

- 1-(2*R*-2-Aminobutanoyl)-4-[*o*-(*o*-hydroxymethylphenylthio)benzyl]semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-bromobenzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(2-chloro-6-fluorobenzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-ethoxybenzyl)semicarbazide;  
5 1-(2*R*-2-Aminobutanoyl)-4-([1,1'-biphenyl-4-yl]methyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-([1,1'-biphenyl-2-yl]methyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*p*-[thiophen-2-yl]benzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-[*N*-morpholinyl]benzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(1-naphthylmethyl)semicarbazide;  
10 1-(2*R*-2-Aminobutanoyl)-4-(*o*-[*N*-piperidiny]benzyl)semicarbazide; and  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-methylthiobenzyl)semicarbazide.

The compounds of the invention may exist as geometric isomers or optical isomers or stereoisomers as well as tautomers. Accordingly, the invention includes all geometric  
15 isomers and tautomers including mixtures and racemic mixtures of these and a pharmaceutically acceptable salt thereof, especially all *R*- and *S*- isomers.

The present invention also encompasses pharmaceutically acceptable salts of the present compounds. Such salts include pharmaceutically acceptable acid addition  
20 salts, pharmaceutically acceptable metal salts, ammonium and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, nitric acids and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic,  
25 benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pantoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, EDTA, glycolic, *p*-aminobenzoic, glutamic, benzenesulfonic, *p*-toluenesulfonic acids and the like. Further examples of pharmaceutically acceptable  
30 inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. 1977, 66, 2, which is incorporated herein by reference. Examples of metal salts include lithium, sodium, potassium, magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium,  
35 hydroxyethylammonium, diethylammonium, butylammonium, tetramethylammonium salts and the like.

Also intended as pharmaceutically acceptable acid addition salts are the hydrates, which the present compounds are able to form.

- 5 The acid addition salts may be obtained as the direct products of compound synthesis. In the alternative, the free base may be dissolved in a suitable solvent containing the appropriate acid, and the salt isolated by evaporating the solvent or otherwise separating the salt and solvent.
- 10 The compounds of the present invention may form solvates with standard low molecular weight solvents using methods well known to the person skilled in the art. Such solvates are also contemplated as being within the scope of the present invention.
- 15 The invention also encompasses prodrugs of the present compounds, which on administration undergo chemical conversion by metabolic processes before becoming active pharmacological substances. In general, such prodrugs will be functional derivatives of the present compounds, which are readily convertible in vivo into the required compound of the Formula I. Prodrugs are any covalently bonded compounds,
- 20 which release the active parent drug according to Formula I in vivo. If a chiral center or another form of an isomeric center is present in a compound of the present invention, all forms of such isomer or isomers, including enantiomers and diastereomers, are intended to be covered herein. Inventive compounds containing a chiral center may be used as a racemic mixture, an enantiomerically enriched mixture, or the racemic
- 25 mixture may be separated using well-known techniques and an individual enantiomer may be used alone. In cases in which compounds have unsaturated carbon-carbon double bonds, both the cis (Z) and trans (E) isomers are within the scope of this invention. In cases wherein compounds may exist in tautomeric forms, such as keto-enol tautomers, each tautomeric form is contemplated as being included within this
- 30 invention whether existing in equilibrium or predominantly in one form. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", ed. H. Bundgaard, Elsevier, 1985.

The invention also encompasses active metabolites of the present compounds.

35

The present invention includes all complexes of the compounds of this invention.



The meaning of any substituent at any one occurrence in Formula I or any subformula thereof is independent of its meaning, or any other substituent's meaning, at any other occurrence, unless specified otherwise.

5

In a preferred embodiment of this invention, the compounds of Formula I exhibit an  $IC_{50}$  value of less than  $500 \mu M$ , preferably less than  $100 \mu M$ , more preferably less than  $50 \mu M$ , even more preferably less than  $1 \mu M$ , especially less than  $500 \text{ nM}$ , particularly less than  $100 \text{ nM}$ , when subjected to a human dipeptidyl dipeptidase-I assay such as the

10

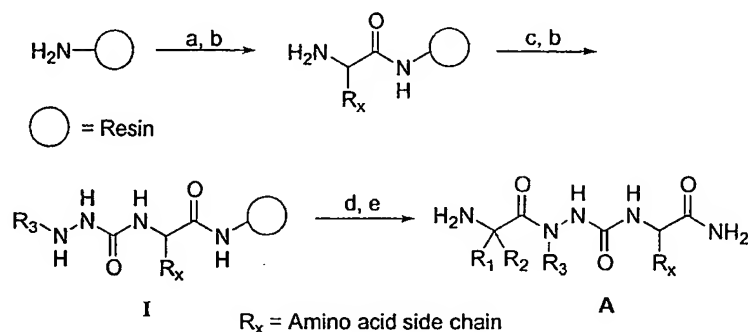
### Methods of Preparation

The compounds of the present invention may be prepared by the methods set forth in the schemes 1-3 below.

15

A list of the used abbreviations is given below under Materials and Methods.

### Scheme 1



20

Reagents and conditions: a) Fmoc-AA-OPfp, DhbtOH, DMF; or Fmoc-AA-OH, TBTU, NEM, DMF; b) piperidine, DMF; c) Fmoc-NHNH-CO<sub>2</sub>Pfp, DhbtOH, DMF; or Fmoc-N(R<sub>3</sub>)NH<sub>2</sub>, CDI, DMF; d) FmocNHC(R<sub>1</sub>)R<sub>2</sub>CO<sub>2</sub>H or, BocNHC(R<sub>1</sub>)R<sub>2</sub>CO<sub>2</sub>H TBTU, NEM, DMF; or, FmocNHC(R<sub>1</sub>)R<sub>2</sub>CO<sub>2</sub>Pfp, DhbtOH, DMF; e) 2% TIPS in TFA.

25

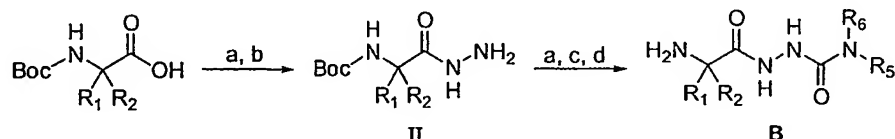
The first Fmoc protected amino acid is loaded onto Rink derivatized resin by conventional peptide coupling procedures (such as by the use of Pfp esters or the TBTU coupling reagent), in an aprotic solvent (such as DMF). The semicarbazide (intermediate I) is formed by acylation with either Fmoc-NHNH-CO<sub>2</sub>Pfp or Fmoc-N(R<sub>3</sub>)NH-COIm in an aprotic solvent (such as DMF), with or without the use of DhbtOH

30

additive. The N-terminal residue is introduced by acylation, such as with an activated Fmoc or Boc protected amino acid, in DCM or DMF, with or without the addition of base. The desired products **A** are obtained upon cleavage with a TFA/TIPS mixture (the Fmoc group, if any, is removed prior to cleavage).

5

### Scheme 2

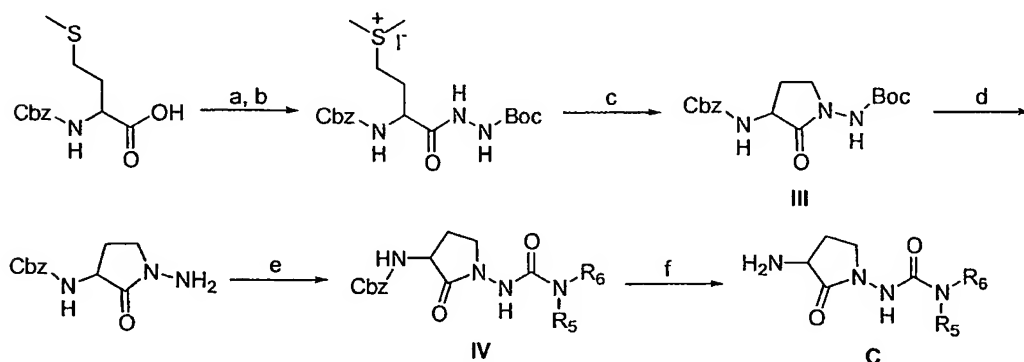


10 Reagents and conditions: a) CDI, THF; b)  $\text{NH}_2\text{NH}_2$ , THF; c)  $\text{R}_5\text{R}_6\text{NH}$ , THF; d) TFA.

Semicarbazides of formula **B** are prepared without the use of solid support. A Boc protected amino acid is converted to its hydrazide by activation with CDI, followed by reaction with hydrazine hydrate. The amino acid hydrazide **II** is then reacted with CDI again, and displacement with a range of different amines, followed by Boc deprotection gives the crude products, which are purified by HPLC.

15

### Scheme 3



20

Reagents and conditions: a) CDI, THF; then  $\text{BocNHNH}_2$ ; b) MeI; c) NaH, DMF, DCM; d) TFA, DCM; or HCl in dioxane; e) CDI, THF; then  $\text{R}_5\text{R}_6\text{NH}$ ; f) TFA, TMSBr, PhSMc; or HBr in acetic acid

25

Semicarbazides of formula **C** are prepared starting from the 3-benzyloxycarbonylamino-1-*tert*-butoxycarbonylaminopyrrolidin-2-one **III**, which is prepared analogous to general procedures described by Freidinger *et al.* (*J. Org.*

*Chem.*, 1982, 47, 104-109) and Duffy *et al.* (*Bioorg. Med. Chem. Lett.*, 1999, 9, 1907-1910). The Boc group is preferably removed with HCl in dioxane. Formation of the semicarbazide bond (intermediate IV) is achieved by reaction with CDI in THF followed by displacement with the appropriate amine. The Cbz group is either removed by  
5 TMSBr in TFA using thioanisole as scavenger, or by HBr in acetic acid. The desired products are obtained after HPLC purification.

The starting materials used herein are commercially available amino acids or are prepared by routine methods well known to those of ordinary skill in the art and can be  
10 found in standard reference books, such as the COMPENDIUM OF ORGANIC SYNTHETIC METHODS, Vol. I-VI (published by Wiley-Interscience).

Coupling methods to form amide bonds herein are generally well known to the art. The methods of peptide synthesis generally set forth by Bodansky *et al.*, THE PRACTICE  
15 OF PEPTIDE SYNTHESIS, Springer-Verlag, Berlin, 1984; E. Gross and J. Meienhofer, THE PEPTIDES, Vol. 1, 1-284 (1979); and J. M. Stewart and J. D. Young, SOLID PHASE PEPTIDE SYNTHESIS, 2d Ed., Pierce Chemical Co., Rockford, Ill., 1984, are generally illustrative of the technique and are incorporated herein by reference.

20 Synthetic methods to prepare the compounds of this invention frequently employ protective groups to mask a reactive functionality or minimize unwanted side reactions. Such protective groups are described generally in Green, T. W. PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York (1981). The term "amino protecting groups" generally refers to the Boc, acetyl, benzoyl, Fmoc and Cbz  
25 groups and derivatives thereof as known to the art. Methods for protection and deprotection, and replacement of an amino protecting group with another moiety are well known.

Acid addition salts of the compounds of Formula I are prepared in a standard manner  
30 in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic, hydrofluoric, sulfuric, phosphoric, acetic, trifluoroacetic, maleic, succinic or methanesulfonic. Certain of the compounds form inner salts or zwitterions that may be acceptable. Cationic salts are prepared by treating the parent compound with an excess of an alkaline reagent, such as a hydroxide, carbonate or  
35 alkoxide, containing the appropriate cation; or with an appropriate organic amine. Cations such as Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup>, Mg<sup>++</sup> and NH<sub>4</sub><sup>+</sup> are specific examples of cations

present in pharmaceutically acceptable salts. Halides, sulfate, phosphate, alkanoates (such as acetate and trifluoroacetate), benzoates, and sulfonates (such as mesylate) are examples of anions present in pharmaceutically acceptable salts.

5 Pharmaceutical compositions

In one aspect of this invention, there is provided a pharmaceutical composition comprising, as an active ingredient, a compound of the present invention together with a pharmaceutically acceptable carrier or diluent. This composition may be in unit dosage form and may comprise from about 0.1  $\mu$ g to about 1000 mg such as, e.g.,  
10 from about 1  $\mu$ g to about 500 mg, from about 5  $\mu$ g to about 250 mg, from about 50  $\mu$ g to about 100 mg or from about 0.1 to about 50 mg, of the compound of the invention or a pharmaceutically acceptable salt or ester thereof. The composition of the invention may be used for oral, nasal, transdermal, pulmonal or parenteral administration. It is contemplated that the pharmaceutical composition of the invention is useful for  
15 treatment of inflammation, type2 diabetes, asthma, severe influenza, respiratory syncytial virus infection, CD8 T cell inhibition, inflammatory bowel diseases, psoriasis, atopic dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease, periodontitis, rheumatoid arthritis, Huntington's disease, Chagas' disease, Alzheimer's disease, sepsis and/or for application in target cell apoptosis.

20

The compounds of the invention may be administered alone or in combination with pharmaceutically acceptable carriers, diluents or excipients, in either single or multiple doses. Accordingly, the compounds of Formula I may be used in the manufacture of a medicament. The pharmaceutical compositions according to the invention may be  
25 formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington: The Science and Practice of Pharmacy, 19.sup.th Edition, Gennaro, Ed., Mack Publishing Co., Easton, Pa., 1995.

30 The pharmaceutical compositions may be specifically formulated for administration by any suitable route such as the oral, rectal, nasal, pulmonary, topical (including buccal and sublingual), transdermal, intracisternal, intraperitoneal, vaginal and parenteral (including subcutaneous, intramuscular, intrathecal, intravenous and intradermal) route, the oral route being preferred. It will be appreciated that the preferred route will depend  
35 on the general condition and age of the subject to be treated, the nature of the condition to be treated and the active ingredient chosen.

Pharmaceutical compositions for oral administration include solid dosage forms such as capsules, tablets, dragees, pills, lozenges, powders and granules. Where appropriate, they can be prepared with coatings such as enteric coatings or they can be formulated so as to provide controlled release of the active ingredient such as sustained or prolonged release according to methods well known in the art.

Liquid dosage forms for oral administration include solutions, emulsions, suspensions, syrups and elixirs.

Pharmaceutical compositions for parenteral administration include sterile aqueous and non-aqueous injectable solutions, dispersions, suspensions or emulsions as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use. Depot injectable formulations are also contemplated as being within the scope of the present invention.

Other suitable administration forms include suppositories, sprays, ointments, cremes, gels, inhalants, dermal patches, implants etc.

A typical oral dosage may be in the range of from about 0.001 to about 100 mg/kg body weight per day, preferably from about 0.01 to about 50 mg/kg body weight per day, and more preferred from about 0.05 to about 10 mg/kg body weight per day administered in one or more dosages such as 1 to 3 dosages. The exact dosage will depend upon the frequency and mode of administration, the sex, age, weight and general condition of the subject treated, the nature and severity of the condition treated and any concomitant diseases to be treated and other factors evident to those skilled in the art. A person skilled in the art know how to adjust the dosage to obtain the desired effect.

The formulations may conveniently be presented in unit dosage form by methods known to those skilled in the art. A typical unit dosage form for oral administration one or more times per day such as 1 to 3 times per day may contain of from about 1 µg to about 1000 mg such as, e.g., from about 0.05 to about 1000 mg, preferably from about 0.1 to about 500 mg, and more preferred from about 0.5 mg to about 200 mg.

For parenteral routes, such as intravenous, intrathecal, intramuscular and similar administration, typically doses are in the order of about half the dose employed for oral administration.

- 5 The compounds of this invention are generally utilized as the free substance or as a pharmaceutically acceptable salt thereof. One example is an acid addition salt of a compound having the utility of a free base. When a compound of the Formula (I) contains a free base such salts are prepared in a conventional manner by treating a solution or suspension of a free base of the Formula (I) with a chemical equivalent of a  
10 pharmaceutically acceptable acid, for example, inorganic and organic acids. Representative examples are mentioned above. Physiologically acceptable salts of a compound with a hydroxy group include the anion of said compound in combination with a suitable cation such as sodium or ammonium ion.
- 15 For parenteral administration, solutions of the novel compounds of the Formula (I) in sterile aqueous solution, aqueous propylene glycol or sesame or peanut oil may be employed. Such aqueous solutions should be suitable buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and  
20 intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art.

- Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. Examples of solid carriers are lactose, terra alba,  
25 sucrose, cyclodextrin, talc, gelatine, agar, pectin, acacia, magnesium stearate, stearic acid or lower alkyl ethers of cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene or water. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.
- 30 The pharmaceutical compositions formed by combining the novel compounds of the Formula (I) and the pharmaceutically acceptable carriers are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules or tablets, each containing a predetermined amount of the active ingredient, and which may include a suitable excipient. These formulations may be in the form of powder or granules, as a solution or suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion.

If a solid carrier is used for oral administration, the preparation may be tableted; placed in a hard gelatine capsule in powder or pellet form or it can be in the form of a troche or lozenge. The amount of solid carrier will vary widely but will usually be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatine capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

A typical tablet, which may be prepared by conventional tableting techniques, may contain:

*Core:*

Active compound (free compound or salt)	5.0 mg
Lactosum Ph. Eur.	67.8 mg
Cellulose, microcryst. (Avicel)	31.4 mg
Amberlite	1.0 mg
Magnesii stearas	q.s.

*Coating:*

Hydroxypropyl methylcellulose approx.	9 mg
Acylated monoglyceride approx.	0.9 mg

If desired, the pharmaceutical composition of the invention may comprise the compound of the Formula (I) in combination with further pharmacologically active substances such as those described in the foregoing.

Use of the invention

The compounds of Formula I are useful as protease inhibitors, particularly as inhibitors of cysteine and serine proteases, more particularly as inhibitors of cysteine proteases, even more particularly as inhibitors of cysteine proteases of the papain superfamily, yet more particularly as inhibitors of DPP-I and selective inhibitors of DPP-I. The present

invention provides useful compositions and formulations of said compounds, including pharmaceutical compositions and formulations of said compounds.

The compounds of the present invention may especially be useful for the treatment or  
5 prevention of diseases such as inflammation, type2 diabetes and similar diseases  
involving a protease. The present compounds are especially useful for treating  
diseases in which cysteine proteases are implicated and especially diseases in which  
dipeptidyl peptidase-I is implicated, most particularly mast cell inflammatory mediated  
diseases. Examples of diseases in which dipeptidyl peptidase-I is implicated are:  
10 inflammation, type2 diabetes, asthma, severe influenza, respiratory syncytial virus  
infection, CD8 T cell inhibition, inflammatory bowel diseases, psoriasis, atopic  
dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease,  
periodontitis, rheumatoid arthritis, Huntington's disease, Chagas' disease, Alzheimer's  
disease, sepsis as well as in target cell apoptosis.

15 Accordingly, in one aspect the present invention relates to a method for the treatment  
of ailments, the method comprising administering to a subject in need thereof an  
effective amount of a compound or a composition of this invention. It is contemplated  
that an effective amount of a compound or a composition of this invention corresponds  
20 to an amount of active ingredient, i.e. active compound or a pharmaceutically  
acceptable salt or ester thereof, in the range of e.g. from about 0.05 to about 100 mg  
per day, preferably from about 0.1 to about 50 mg per day.

In yet another aspect, the present invention relates to use of a compound of this  
25 invention for the preparation of a medicament, preferably a medicament for treatment  
of inflammation, type2 diabetes, asthma, inflammatory bowel diseases, psoriasis,  
atopic dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease,  
periodontitis, arthritis, Huntington's disease, Chagas' disease, Alzheimer's disease,  
sepsis or for application in target cell apoptosis.

30 For acute therapy, parenteral administration of a compound of Formula I is preferred.  
An intravenous infusion of the compound in 5% dextrose in water or normal saline, or a  
similar formulation with suitable excipients, is most effective, although an intramuscular  
bone injection is also useful. Typically, the parenteral dose will be about 0.01 to about  
35 100 mg/kg; preferably between 0.1 and 20 mg/kg, in a manner to maintain the  
concentration of drug in the plasma at a concentration effective to inhibit dipeptidyl



dipeptidase-I (cathepsin C). The compounds may be administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about 400 mg/kg/day. The precise amount of an inventive compound which is therapeutically effective, and the route by which such compound is best administered, is readily determined by one of  
5 ordinary skill in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect.

The compounds of this invention may also be administered orally to the patient, in a manner such that the concentration of drug is sufficient to inhibit bone resorption or to  
10 achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the condition of the patient. Preferably the oral dose would be about 0.5 to about 20 mg/kg.

15 No unacceptable toxicological effects are expected when compounds of the present invention are administered in accordance with the present invention.

The compounds of the present invention fully or partly inhibit dipeptidyl-peptidase I, and are thus useful for the treatment and/or prevention of a wide variety of conditions and  
20 disorders in which inhibition of DPP-I is beneficial. It is of specific interest that a selective inhibitory effect can be obtained by use of the compounds of the invention, i.e. side-effects relating to inhibition of other proteases can be markedly reduced or eliminated.

25 Accordingly, in another aspect the present invention relates to a compound of the general Formula (I) or any optical or geometric isomer or tautomeric form thereof including mixtures of these or a pharmaceutically acceptable salt thereof for use as a pharmaceutical composition.

30 The invention also relates to pharmaceutical compositions comprising, as an active ingredient, at least one compound of the Formula (I) or any optical or geometric isomer or tautomeric form thereof including mixtures of these or a pharmaceutically acceptable salt thereof together with one or more pharmaceutically acceptable carriers or diluents.

35 Furthermore, the invention also relates to the use of the compounds and compositions of the present invention to modulate DPPI levels in a subject (e.g., human) in need

thereof in an amount effective to modulate DPPI levels. In a preferred embodiment, the compound or composition inhibits DPPI.

In the following synthetic examples, all of the starting materials were obtained from commercial sources unless otherwise indicated. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. These examples are given to illustrate the invention, not to limit its scope.

## 10 EXAMPLES

### Materials and Methods

Temperature is in degrees Centigrade (°C). Experiments were conducted at room temperature (20 °C), unless otherwise noted. All solvents were HPLC grade.

15 Anhydrous solvents were obtained by storing over 4 Å activated molecular sieves. Unless otherwise noted starting materials were purchased from commercial suppliers and used without further purification.

Solid phase reactions run at room temperature were performed in flat-bottom polyethylene syringes equipped with sintered Teflon filters (70 µm pores), Teflon tubing, Teflon valves for flow control, and suction to drain the syringes from below, or on a VacMaster parallel synthesis rack. Fmoc deprotection was performed with 20% (v/v) piperidine in DMF (2 + 10 min.). TBTU-couplings were performed by dissolving the acid (3 eq.) in DMF with NEM (4 eq.), followed by addition of TBTU (2.88 eq.). The resulting solution was preactivated for 10 min. before use. Pfp esters (3 eq.) were coupled with DhbtOH (1 eq.) present. The disappearance of the bright yellow color indicated complete capping of the resin-bound amino groups. Solid phase reactions were generally run in an amount of solvent that was enough to cover the resin. Resin loadings were determined by Fmoc cleavage and optical density measurements at 290 nm, using a calibration curve.

NMR data were acquired on a Bruker Advance DRX 250. CDCl<sub>3</sub> is deuteriochloroform, DMSO-d<sub>6</sub> is hexadeuteriodimethylsulfoxide, D<sub>2</sub>O is deuteriooxide, and CD<sub>3</sub>OD is tetradeuteriomethanol. Abbreviation for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, app = apparent, br = broad. Chemical shifts are reported

in ppm, relative to internal solvent peaks (2.49 for DMSO-*d*<sub>6</sub>, 7.25 for CDCl<sub>3</sub>, 4.75 for D<sub>2</sub>O, 3.35 for CD<sub>3</sub>OD). Coupling constants *J* are reported in Hz. ES-MS spectra were obtained on a Micromass Quattro micro<sup>TM</sup> instrument in the positive mode unless otherwise noted.

5

Analytical HPLC was performed on a Gilson system (UV/VIS-155 detector at 215 and 254 nm, 402 syringe pump, 819 injection module, valvemate 35, 864 degasser, 233 XL on-line column switching module, and a Zorbax 300SB RP-18 column, 4.6 x 50 mm with a 322 pump). Eluents A (0.1% TFA in water) and B (1% TFA in acetonitrile) were used in a linear gradient (0% B → 100% B in 7 min.). Purity (given in parentheses) is at 215 nm.

10

Preparative HPLC was performed on the same Gilson system, using a Zorbax 300SB RP-18, 21.2 mm x 25 cm column, with a flow of 15 mL/min.

15

#### Abbreviations

AA	Amino acid
AFC	7-Amino-trifluoromethyl coumarin
CDI	1,1'-Carbonyldiimidazole
20 DCM	Dichloromethane
DE	Diethylether
DhbtOH	3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine
DIC	<i>N,N'</i> -Diisopropylcarbodiimide
DIPEA	<i>N,N</i> -Diisopropylethylamine
25 DMAP	4-Dimethylaminopyridine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
EA	Ethyl acetate
EDTA	Ethylenediaminetetraacetic acid
30 Fmoc	9-Fluorenylmethoxycarbonyl
Im	1-Imidazolyl
NEM	<i>N</i> -Ethylmorpholine
PE	Petroleum ether (bp. 60-80°)
PEGA	Poly(ethylene glycol)-poly(acryl amide) copolymer
35 Pfp	Pentafluorophenyl

Rink amide linker	<i>p</i> -[( <i>R,S</i> )- $\alpha$ -[1-(9 <i>H</i> -fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid
TBTU	<i>N</i> -[(1 <i>H</i> -Benzotriazol-1-yl)-(dimethylamino)-methylene]- <i>N</i> -methylmethan-aminium tetrafluoroborate <i>N</i> -oxide
5 TFA	Trifluoroacetic acid
THF	Tetrahydrofurane
TIPS	Triisopropylsilane
TMSBr	Trimethylsilyl bromide

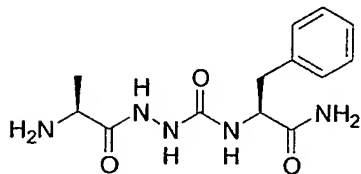
## 10 Nomenclature

The compound class described in the following can be viewed as both azapeptides and derivatives of semicarbazide. In naming the compounds in the examples below the term semicarbazide has been used as the parent group, rather than the term azapeptide.

15

### EXAMPLE 1

#### 1-(2*S*-2-Aminopropionyl)-4-(2*S*-3-phenylpropan-2-yl-amide)semicarbazide



- 20 a) Preparation of Fmoc-NHNH<sub>2</sub>: Fmoc-Cl (10 g, 38.7 mmol) dissolved in diethyl ether (180 mL) was added dropwise to a solution of hydrazine hydrate (3.8 mL, 77 mmol) in diethyl ether (100 mL) cooled in an ice-bath. White precipitation formed quickly. When all the Fmoc-Cl was added the resulting white suspension was warmed to rt. and stirred for 30 min. The white solid was isolated by filtration, washed with diethyl ether
- 25 (x3) and water (x3) and dried *in vacuo* to give the desired product. Yield: 8 g (81%); *R*<sub>f</sub> = 0.2 (PE:EA 1:1 + a few drops of acetic acid); HPLC: *R*<sub>t</sub> = 4.16 min. (>99%); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 250 MHz)  $\delta$  8.34 (br, 1H), 7.90-7.88 (d, *J* = 7.3, 2H), 7.71-7.68 (d, *J* = 7.3, 2H), 7.45-7.29 (m, 4H), 4.30-4.18 (m, 3H), 4.08 (br, 2H); <sup>13</sup>C (DMSO-*d*<sub>6</sub>, 250 MHz)  $\delta$  158.2, 143.8, 140.7, 127.6, 127.0, 125.2, 120.0, 65.6, 46.7; ES-MS: mass calcd for
- 30 C<sub>15</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub> 255.1 (MH<sup>+</sup>). Found *m/z* 255.1.

b) Preparation of Fmoc-NHNH-CO<sub>2</sub>Pfp: Fmoc-NHNH<sub>2</sub> (750 mg, 2.95 mmol) and bis(pentafluorophenyl)carbonate (1221 mg, 3.1 mmol) were stirred in dry THF (15 mL)

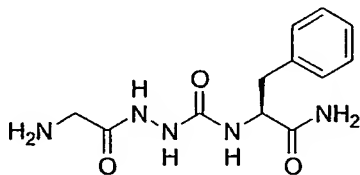
at rt for 18 h. The reaction mixture was diluted with water (50 mL) and extracted with EA (x3). The combined organic fractions were washed with 1 M HCl (x1) and brine (x1), dried (MgSO<sub>4</sub>), and concentrated to give a clear oil, which solidified *in vacuo*. Off-white solid. Yield: 1.32 g (96%). R<sub>f</sub> = 0.4 (PE:EA 3:1). This compound was used crude,  
 5 since it was partially unstable to purification by both recrystallization and silica chromatography.

c) Scheme 1: PEGA<sub>800</sub> resin derivatized with Rink amide linker (L = 0.3 mmol/g, 405 mg, 109 μmol) was Fmoc deprotected, and then washed with DMF (x5). Fmoc-Phe-  
 10 OPfp (182 mg, 328 μmol) and DhbtOH (18 mg, 109 μmol) were dissolved in DMF (3 mL) and added to the resin. After disappearance of the yellow color the resin was washed with DMF (x5). Following Fmoc deprotection (as above) and wash with DMF (x5) the resin-bound amine was acylated with Fmoc-NHNH-CO<sub>2</sub>Pfp (170 mg, 366 μmol) in the presence of DhbtOH (18 mg, 109 μmol) in DMF (3 mL) for 18 h. The resin  
 15 was washed with DMF (x5), Fmoc deprotected and washed again with DMF (x5). Fmoc-Ala-OPfp (174 mg, 328 μmol) and DhbtOH (18 mg, 109 μmol) were dissolved in DMF (3 mL) and added to the resin. After disappearance of the yellow color the resin was washed with DMF (x5), then Fmoc deprotected and finally washed with DMF (x5) and DCM (x3). Cleavage was achieved by 1 h treatment with 2% TIPS in TFA (drops of  
 20 DCM added to obtain a homogeneous solution). The resin was washed several times (x6) with TFA solution and the combined washings were concentrated to give a clear oil. The crude product was purified by preparative HPLC. The title compound was obtained as a white solid. Yield: 24.5 mg (69%); HPLC: R<sub>t</sub> = 2.77 min. (>98%); <sup>1</sup>H-NMR (D<sub>2</sub>O, 250 MHz) δ 7.13-6.99 (m, 5H), 4.19-4.13 (dd, J = 6.1, 8.3, 1H), 3.87-3.79 (q, J =  
 25 7.1, 1H), 2.90-2.82 (dd, J = 6.1, 14.0, 1H), 2.75-2.66 (dd, J = 8.4, 14.0, 1H), 1.26-1.23 (d, J = 7.1, 3H); ES-MS: mass calcd for C<sub>13</sub>H<sub>20</sub>N<sub>5</sub>O<sub>3</sub> 294.1 (MH<sup>+</sup>). Found m/z 294.2.

## EXAMPLE 2

### 1-(Aminoacetyl)-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide

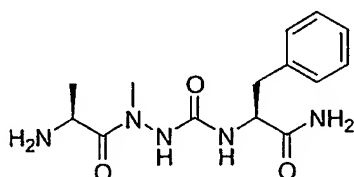
30



Scheme 1: Starting from 200 mg resin (0.06 mmol), following the general procedure of Example 1c, except substituting Fmoc-Ala-OPfp for Fmoc-Gly-OPfp. Formation of the

semicarbazide bond was achieved by acylation with Fmoc-NHNHCOIm. This was prepared just prior to use by reaction of FmocNHNH<sub>2</sub> (0.18 mmol) and CDI (0.18 mmol) in dry DMF (1 mL) for 1h. The resulting solution was then added directly to the resin. Reaction time 16 h. The title compound was obtained as a white solid. Yield: 3.1 mg (19%); HPLC: R<sub>t</sub> = 2.42 min. (>99%); ES-MS: mass calcd for C<sub>12</sub>H<sub>18</sub>N<sub>5</sub>O<sub>3</sub> 280.1 (MH<sup>+</sup>). Found *m/z* 280.1

## EXAMPLE 3

1-(2S-2-Aminopropionyl)-1-methyl-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide

Starting from 315 mg resin (0.095 mmol), following the general procedure of Example 2, except substituting FmocNHNH<sub>2</sub> for FmocN(Me)NH<sub>2</sub> (prepared according to procedure in *J. Org. Chem.*, **1999**, 64, 7388-7394). Preactivation with CDI performed for 3 h instead of just 1 h. White solid. Yield: 13.3 mg (46%); HPLC: R<sub>t</sub> = 2.71 min. (>99%); <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>, 250 MHz) δ 8.64 (s, 1H), 8.10 (br, 2H), 7.59 (s, 1H), 7.31-7.14 (m, 6H), 6.88 (br, 1H), 4.43-4.35 (m, 1H), 3.50 (1H, hidden under water signal), 3.09-3.05 (m, 1H), 2.90 (s, 3H), 2.84-2.74 (m, 1H), 1.30-1.18 (br, 3H); ES-MS: mass calcd for C<sub>14</sub>H<sub>22</sub>N<sub>5</sub>O<sub>3</sub> 308.2 (MH<sup>+</sup>). Found *m/z* 308.0.

20

Examples 4-26 were all prepared using TentaGel S-NH<sub>2</sub> resin (L = 0.41 mmol/g, 150 mg, 0.062 mmol) derivatized with the Rink linker. Coupling of individual amino acid derivatives were performed using TBTU or Pfp-ester chemistry, as described in Example 1 and in Materials and Methods. The semicarbazide bond was formed using Fmoc-NHNH-COIm, as described in Example 2. Wash, cleavage and purification by HPLC were performed as outlined in Example 1c.

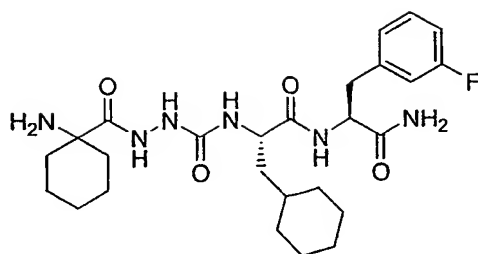
25

## EXAMPLE 4

1-(1-Aminocyclohexane-1-carbonyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-cyclohexylpropan-2-yl-amide]semicarbazide

30

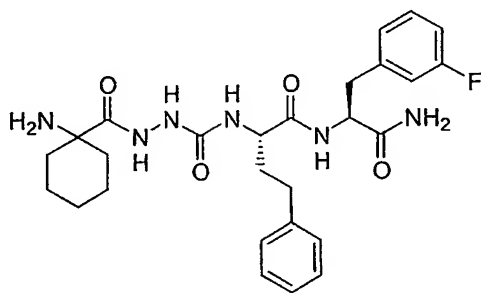
34



White solid. Yield: 19.9 mg (62%); HPLC:  $R_t = 4.10$  min. (>99%);  $^1\text{H-NMR}$  (DMSO- $d_6/\text{D}_2\text{O}$  10:1, 250 MHz)  $\delta$  7.32-7.23 (m, 1H), 7.05-6.94 (m, 3H), 4.46-4.37 (m, 1H), 4.07-4.01 (m, 1H), 3.06-2.98 (dd,  $J = 5.0, 13.8$ , 1H), 2.86-2.77 (dd,  $J = 9.2, 13.8$ , 1H),  
 5 2.05-1.96 (m, 2H), 1.74-1.15 (m, 19H), 1.07-0.74 (m, 2H); ES-MS: mass calcd for  $\text{C}_{26}\text{H}_{40}\text{FN}_6\text{O}_4$  519.3 ( $\text{MH}^+$ ). Found  $m/z$  519.1.

## EXAMPLE 5

1-(1-Aminocyclohexane-1-carbonyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-  
 10 amide]-4-phenylbutan-2-yl-amide]semicarbazide

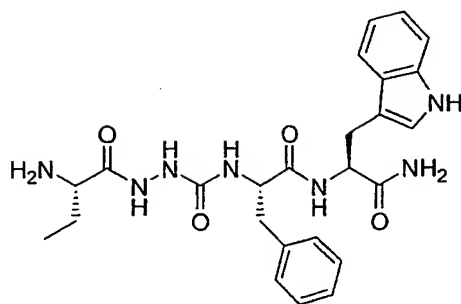


White solid. Yield: 16.9 mg (52%); HPLC:  $R_t = 4.21$  min. (>99%);  $^1\text{H-NMR}$  (DMSO- $d_6/\text{D}_2\text{O}$  10:1, 250 MHz)  $\delta$  7.33-6.95 (m, 9H), 4.50-4.44 (m, 1H), 4.13-4.07 (m, 1H),  
 15 3.09-3.02 (dd,  $J = 5.0, 13.8$ , 1H), 2.88-2.79 (dd,  $J = 9.7, 13.8$ , 1H), 2.53-2.44 (2H, partially under DMSO signal), 2.07-1.99 (m, 2H), 1.87-1.14 (m, 10H); ES-MS: mass calcd for  $\text{C}_{27}\text{H}_{36}\text{FN}_6\text{O}_4$  527.3 ( $\text{MH}^+$ ). Found  $m/z$  527.0.

## EXAMPLE 6

20 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide

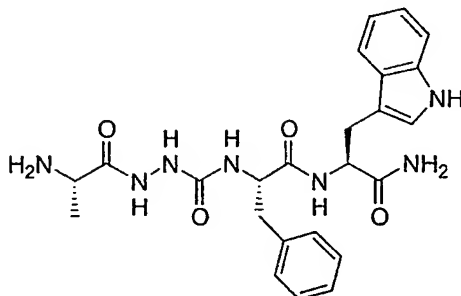
35



White solid. Yield: 13.3 mg (44%); HPLC:  $R_t = 3.64$  min. (>99%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  7.66-7.63 (d,  $J = 7.6$ , 1H), 7.52-7.49 (d,  $J = 8.0$ , 1H), 7.34-7.13 (m, 6H), 7.01-6.97 (m, 2H), 4.61-4.56 (m, 1H), 4.35-4.29 (t,  $J = 7.4$ , 1H), 4.02-3.96 (t,  $J = 6.6$ , 1H),  
 5 3.36-3.28 (dd,  $J = 5.9$ , 14.7, 1H), 3.19-3.10 (dd,  $J = 8.4$ , 14.7, 1H), 2.88-2.77 (m, 2H), 1.99-1.87 (m, 2H), 1.04-0.98 (t,  $J = 7.6$ , 3H); ES-MS: mass calcd for  $\text{C}_{25}\text{H}_{32}\text{N}_7\text{O}_4$  494.2 ( $\text{MH}^+$ ). Found  $m/z$  494.0.

## EXAMPLE 7

10 1-(2S-2-Aminopropanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide

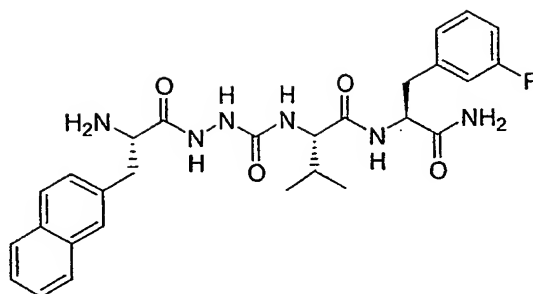


White solid. Yield: 13.1 mg (44%); HPLC:  $R_t = 3.56$  min. (>99%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  7.69-7.66 (d,  $J = 7.6$ , 1H), 7.42-7.39 (d,  $J = 7.9$ , 1H), 7.31-7.02 (m, 8H), 4.56-4.41 (m, 2H), 3.91-3.75 (1H, partially under water signal), 3.24-3.16 (dd,  $J = 5.3$ , 14.6, 1H), 3.09-2.99 (m, 2H), 2.86-2.77 (dd,  $J = 8.1$ , 13.8, 1H), 1.44-1.41 (d,  $J = 7.0$ , 3H; minor isomeric signal observed); ES-MS: mass calcd for  $\text{C}_{24}\text{H}_{30}\text{N}_7\text{O}_4$  480.2 ( $\text{MH}^+$ ). Found  $m/z$  480.1.

## EXAMPLE 8

20 1-(2S-3-Naphthyl-2-propanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide]semicarbazide

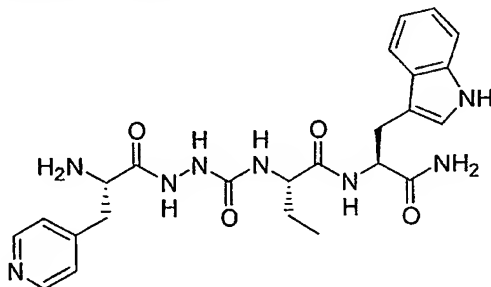




White solid. Yield: 20.5 mg (62%); HPLC:  $R_t = 4.11$  min. (>95%);  $^1\text{H-NMR}$  (DMSO- $d_6/\text{D}_2\text{O}$  10:1, 250 MHz)  $\delta$  7.92-7.81 (m, 4H), 7.34-7.31 (d,  $J = 7.9$ , 1H), 7.55-7.43 (m, 3H), 7.34-7.25 (m, 1H), 7.10-6.95 (m, 3H), 4.50-4.45 (m, 1H), 4.13-4.08 (m, 1H), 4.03-4.01 (d,  $J = 6.0$ , 1H), 3.36-3.28 (dd,  $J = 5.7, 14.4$ , 1H), 3.21-3.13 (dd,  $J = 7.5, 14.4$ , 1H), 3.06-2.95 (dd,  $J = 5.3, 13.8$ , 1H), 2.88-2.79 (dd,  $J = 9.4, 13.8$ , 1H), 1.99-1.86 (m, 1H), 0.80-0.71 (dd,  $J = 6.6$ , 6H); ES-MS: mass calcd for  $\text{C}_{28}\text{H}_{34}\text{FN}_6\text{O}_4$  537.3 ( $\text{MH}^+$ ). Found  $m/z$  537.0.

#### 10 EXAMPLE 9

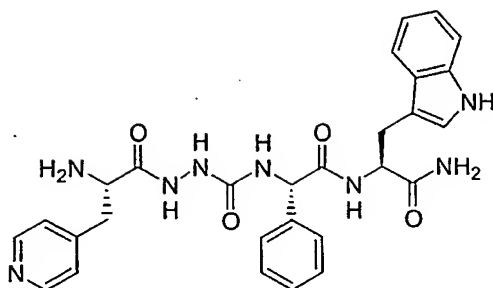
1-[2S-2-Amino-3-(4-pyridyl)propanoyl]-4-[2S-N-(2S-3-(indol-3-yl)propan-2-yl-amide)-butan-2-yl-amide]semicarbazide



White solid. Yield: 13.8 mg (45%); HPLC:  $R_t = 2.91$  min. (>99%);  $^1\text{H-NMR}$  (DMSO- $d_6/\text{D}_2\text{O}$  10:1, 250 MHz)  $\delta$  8.79-8.76 (d,  $J = 6.6$ , 2H), 7.87-7.84 (d,  $J = 6.6$ , 2H), 7.62-7.59 (d,  $J = 7.5$ , 1H), 7.34-7.31 (d,  $J = 6.9$ , 1H), 7.13-6.95 (m, 3H), 4.51-4.45 (m, 1H), 4.20-4.14 (m, 1H), 4.09-4.04 (dd,  $J = 5.3, 7.5$ , 1H), 3.41-3.20 (m, 2H), 3.17-3.09 (dd,  $J = 5.3, 14.8$ , 1H), 3.01-2.92 (dd,  $J = 8.2, 14.8$ , 1H), 1.70-1.40 (m, 2H), 0.81-0.75 (t,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $\text{C}_{24}\text{H}_{30}\text{N}_8\text{O}_4$  495.2 ( $\text{MH}^+$ ). Found  $m/z$  495.0.

#### EXAMPLE 10

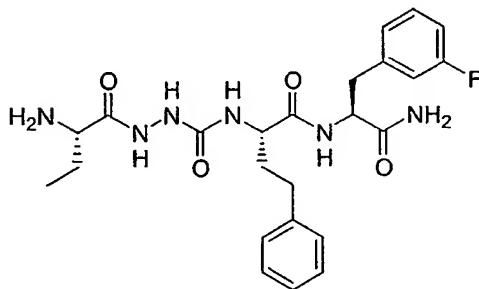
1-[2S-2-Amino-3-(4-pyridyl)propanoyl]-4-[2S-N-(2S-3-(indol-3-yl)propan-2-yl-amide)-2-phenylethan-2-yl-amide]semicarbazide



White solid. Yield: 18.9 mg (57%); HPLC:  $R_t = 3.26$  min. (>36%)/3.36 min. (>63%); isobaric peaks;  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6/\text{D}_2\text{O}$  10:1, 250 MHz)  $\delta$  8.80-8.73 (m, 2H), 7.89-7.84 (m, 2H), 7.62-7.54 (m, 1H), 7.36-6.88 (m, 9H), 5.38 (s, 0.5H), 5.32 (s, 0.5H), 4.53-4.37 (m, 1H), 4.21-4.15 (m, 1H), 3.42-2.86 (m, 4H); ES-MS: mass calcd for  $\text{C}_{28}\text{H}_{31}\text{N}_8\text{O}_4$  543.2 ( $\text{MH}^+$ ). Found  $m/z$  543.0.

#### EXAMPLE 11

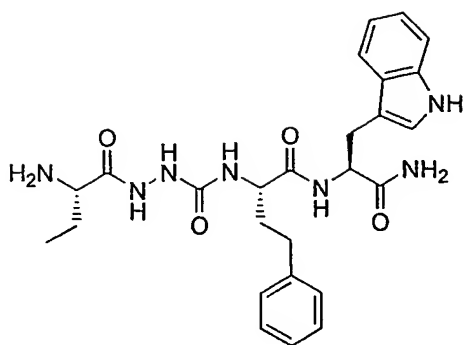
1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide



White solid. Yield: 21.6 mg (54%); HPLC:  $R_t = 4.17$  min. (>99%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  7.22-6.72 (m, 9H), 4.53-4.45 (m, 1H), 3.92-3.81 (m, 2H), 3.15-3.07 (dd,  $J = 5.3$ , 14.4, 1H), 2.85-2.75 (dd,  $J = 10.4$ , 14.4, 1H), 2.32-2.26 (t,  $J = 7.8$ , 2H), 1.88-1.59 (m, 4H), 0.92-0.81 (t,  $J = 7.5$ , 3H); ES-MS: mass calcd for  $\text{C}_{24}\text{H}_{31}\text{FN}_6\text{O}_4$  487.24 ( $\text{MH}^+$ ). Found  $m/z$  487.2

#### EXAMPLE 12

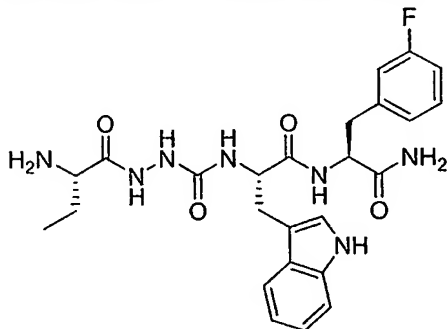
1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide



White solid. Yield: 14 mg (44%); HPLC:  $R_t = 4.03$  min. (>98%);  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 250 MHz)  $\delta$  7.74-7.55 (d,  $J = 7.0$ , 1H), 7.33-7.00 (m, 8H), 4.79-4.70 (m, 1H), 4.17-4.12 (dd,  $J = 5.3$ , 8.2, 1H), 3.88-3.83 (t,  $J = 6.6$ , 1H), 3.43-3.32 (m, 1H, partially hidden under  
 5 methanol signal), 3.22-3.12 (dd,  $J = 8.6$ , 14.7, 1H), 2.58-2.49 (t,  $J = 8.5$ , 2H), 2.05-1.69 (m, 4H), 1.14-1.08 (t,  $J = 7.5$ , 3H); ES-MS: mass calcd for  $\text{C}_{26}\text{H}_{34}\text{N}_7\text{O}_4$  508.3 ( $\text{MH}^+$ ). Found  $m/z$  508.5.

## EXAMPLE 13

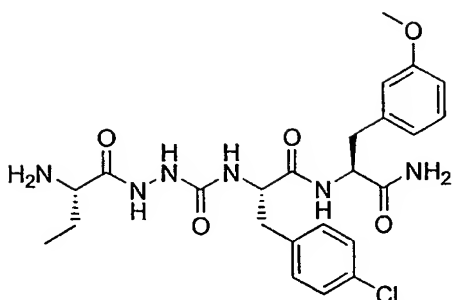
- 10 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide]semicarbazide



- White solid. Yield: 13 mg (41%); HPLC:  $R_t = 3.97$  min. (>99%);  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 250 MHz)  $\delta$  7.73-7.70 (d,  $J = 7.3$ , 1H), 7.49-7.03 (m, 8H), 4.75-4.68 (m, 1H), 4.63-4.58 (t,  $J = 7.0$ , 1H), 3.93-3.88 (m, 1H), 3.35-3.17 (m, 3H), 3.04-2.95 (dd,  $J = 8.4$ , 13.8, 1H),  
 15 2.11-1.92 (m, 2H), 1.23-1.15 (t,  $J = 7.5$ , 3H); ES-MS: mass calcd for  $\text{C}_{25}\text{H}_{31}\text{FN}_7\text{O}_4$  512.3 ( $\text{MH}^+$ ). Found  $m/z$  512.4

## EXAMPLE 14

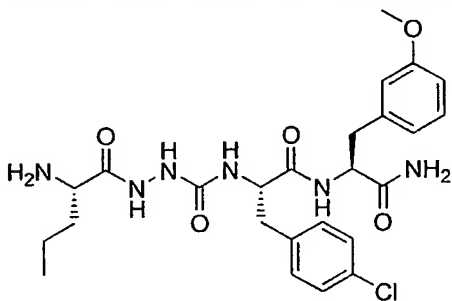
- 20 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide



White solid. Yield: 12 mg (38%); HPLC:  $R_t = 4.14$  min. (>96%);  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 250 MHz; one signal hidden under water peak)  $\delta$  7.41-7.18 (m, 5H), 6.98-6.89 (m, 4H), 4.78-4.70 (m, 1H), 4.62-4.44 (dd,  $J = 5.6, 7.8$ , 1H), 3.33-2.91 (m, 4H), 2.17-1.88 (m, 2H), 1.29-1.18 (t,  $J = 7.6$ , 3H); ES-MS: mass calcd for  $\text{C}_{24}\text{H}_{32}\text{ClN}_6\text{O}_5$  519.2 ( $\text{MH}^+$ ).  
 Found  $m/z$  519.4.

## EXAMPLE 15

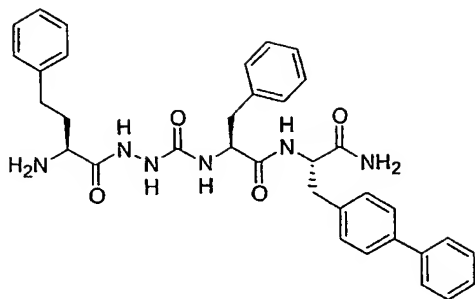
1-(2S-2-Aminopentanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide



White solid. Yield: 18 mg (33%); HPLC:  $R_t = 4.21$  min. (>98%); ES-MS: mass calcd for  $\text{C}_{25}\text{H}_{34}\text{ClN}_6\text{O}_5$  533.2 ( $\text{MH}^+$ ). Found  $m/z$  533.4.

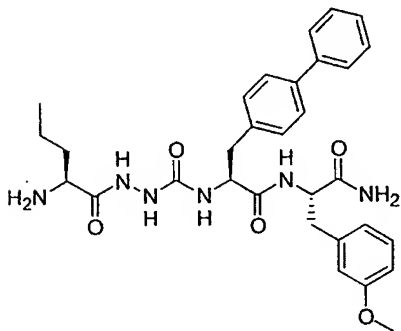
## 15 EXAMPLE 16

1-(2S-2-amino-4-phenylbutanoyl)-4-[2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide



## EXAMPLE 17

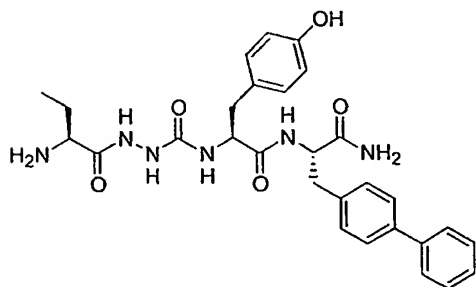
- 5 1-(2S-2-aminopentanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide



10

## EXAMPLE 18

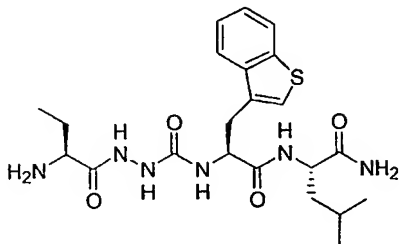
- 10 1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(*p*-hydroxyphenyl)propan-2-yl-amide]semicarbazide



15

## EXAMPLE 19

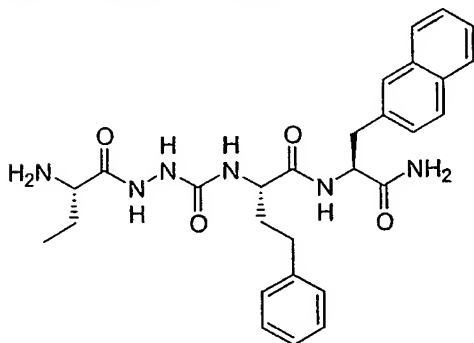
1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-4-methylpentan-2-yl-amide]-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]semicarbazide



5

## EXAMPLE 20

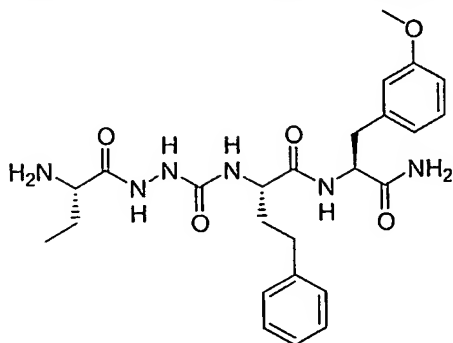
1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide



10

## EXAMPLE 21

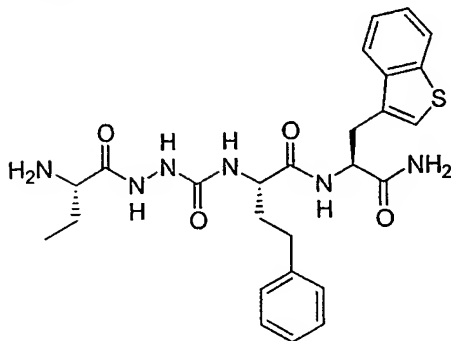
1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide



15

## EXAMPLE 22

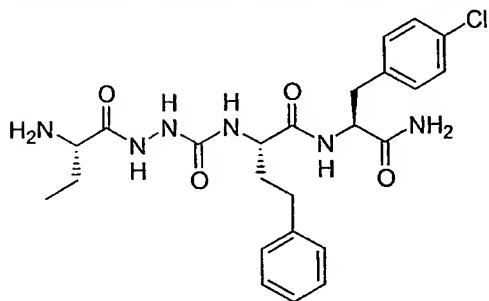
1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide



5

## EXAMPLE 23

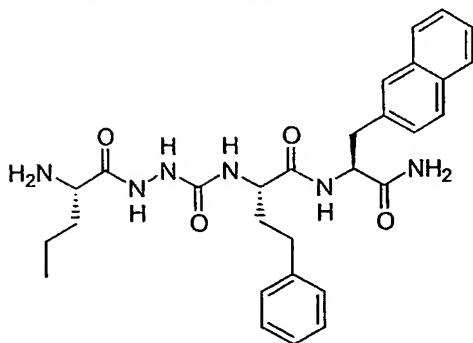
1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(p-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide



10

## EXAMPLE 24

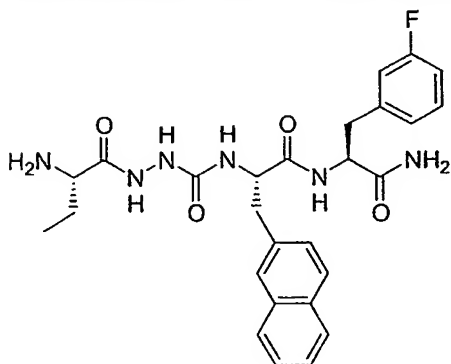
1-(2S-2-Aminopentanoyl)-4-[2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide



15

## EXAMPLE 25

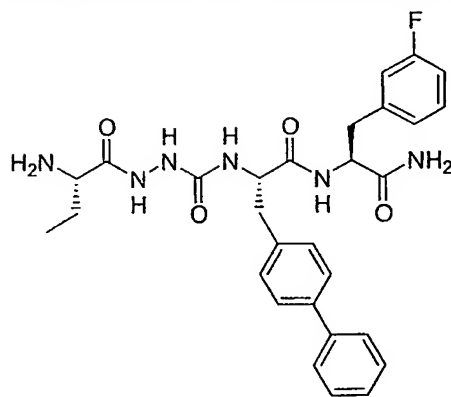
1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide]semicarbazide



5

## EXAMPLE 26

1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide

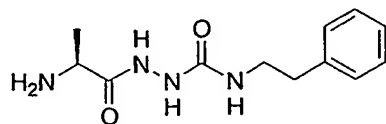


10

## EXAMPLE 27

1-(2S-2-Aminopropionyl)-4-(2-phenylethyl)semicarbazide

15



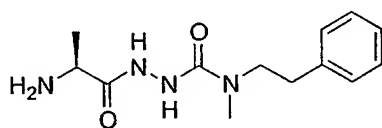
Scheme 2: a) Preparation of *N*<sup>ε</sup>-Boc-L-alaninehydrazide: CDI (4.71 g, 29.1 mmol) was added slowly to a solution of *N*<sup>ε</sup>-Boc-L-alanine (5.0 g, 26.4 mmol) in 50 mL dry THF.



- After addition the solution was stirred for 30 min. Then, hydrazine monohydrate (3.85 mL, 79.3 mmol) was added and the resulting solution was stirred overnight. The reaction mixture was concentrated and the white solid was dissolved in 1,4-dioxane and lyophilized overnight. The crude product was suspended in diethyl ether, cooled to
- 5 -18 °C and filtered. The filtrate was dried *in vacuo* overnight yielding 5.6 g (104%) of crude product. The solid was a mixture of imidazole and desired product (imidazole:product 2:1, determined by <sup>1</sup>H-NMR) and was used without further purification. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub> exchanged with D<sub>2</sub>O, 250 MHz) δ 3.96-3.85 (m, 1H), 1.39-1.31 (s, 6H), 1.18-1.10 (d, *J* = 7.2, 3H)
- 10 b) CDI (44 mg, 0.27 mmol) was added to a solution of *N*<sup>ε</sup>-Boc-L-alaninehydrazide (59.9 mg, 0.295 mmol) in 1.0 mL dry THF. The resulting solution was stirred overnight and 2-phenylethylamine (0.27 mmol) dissolved in 0.5 mL dry THF was added. The reaction mixture was stirred overnight, and then concentrated. The residue was treated with 1 mL 95% TFA/H<sub>2</sub>O for 5 min, and concentrated followed by purification by preparative
- 15 HPLC. The title compound was obtained as a white solid. Yield: 45 mg (66%); HPLC: *R*<sub>t</sub> = 3.11 min. (>99%); <sup>1</sup>H-NMR (D<sub>2</sub>O, 250 MHz) δ (rotamerism observed) 7.37-7.21 (m, 5H), 4.10-4.02 (q, *J* = 7.2, 1H), 3.40-3.31 (td, *J* = 6.9, 2.51, 2H), 2.79-2.72 (t, *J* = 6.9, 2H), 1.50-1.45 (d, *J* = 7.2, 3H); ES-MS: mass calcd for C<sub>12</sub>H<sub>18</sub>N<sub>4</sub>O<sub>2</sub> 251.1 (MH<sup>+</sup>). Found *m/z* 251.0.

## 20 EXAMPLE 28

### 1-(2S-2-Aminopropionyl)-4-(2-phenylethyl)-4-methylsemicarbazide

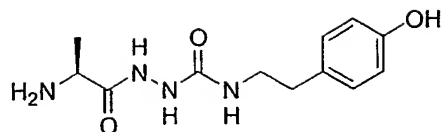


- Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for *N*-methylphenethylamine. The title compound was obtained as a
- 25 white solid. Yield: 29 mg (41%); HPLC: *R*<sub>t</sub> = 3.17 min. (>99%); <sup>1</sup>H-NMR (D<sub>2</sub>O, 250 MHz) δ (rotamerism observed) 7.37-7.20 (m, 5H), 4.15-4.04 (q, *J* = 7.2, 1H), 3.54-3.45 (m, 2H), 2.87-2.75 (m, 5H), 1.54-1.49 (d, *J* = 7.2, 3H); ES-MS: mass calcd for C<sub>13</sub>H<sub>21</sub>N<sub>4</sub>O<sub>2</sub> 265.2 (MH<sup>+</sup>). Found *m/z* 265.0.

30

## EXAMPLE 29

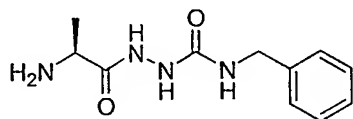
### 1-(2S-2-Aminopropionyl)-4-[2-(*p*-hydroxyphenyl)ethyl]semicarbazide



- Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for tyramine (dissolved in DMF). The title compound was obtained as a white solid. Yield: 20 mg (28%); HPLC:  $R_t = 2.46$  min. (>99%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 7.15-7.07 (m, 2H), 6.84-6.76 (m, 2H), 4.10-4.01 (q,  $J = 7.2$ , 1H), 3.35-3.25 (td,  $J = 6.9$ , 3.14, 2H), 2.71-2.63 (t, 6.9, 2H), 1.50-1.43 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $\text{C}_{12}\text{H}_{19}\text{N}_4\text{O}_3$  267.1 ( $\text{MH}^+$ ). Found  $m/z$  267.0.

## EXAMPLE 30

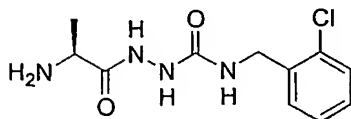
- 10 1-(2S-2-Aminopropionyl)-4-benzylsemicarbazide



- Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for benzylamine. The title compound was obtained as a white solid. Yield: 23 mg (36%); HPLC:  $R_t = 2.57$  min. (>99%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 7.40-7.23 (m, 5H), 4.34-4.29 (s, 2H), 4.15-4.05 (q,  $J = 7.2$ , 1H), 1.55-1.49 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $\text{C}_{11}\text{H}_{17}\text{N}_4\text{O}_2$  237.1 ( $\text{MH}^+$ ). Found  $m/z$  237.0.

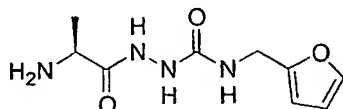
## EXAMPLE 31

- 20 1-(2S-2-Aminopropionyl)-4-(o-chlorobenzyl)semicarbazide



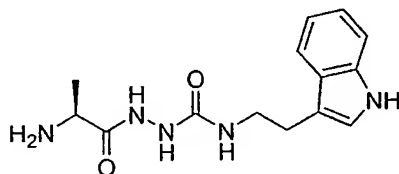
- Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for o-chlorobenzylamine. The title compound was obtained as a white solid. Yield: 24 mg (33%); HPLC:  $R_t = 3.02$  min. (>99%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 7.23-7.01 (m, 4H), 4.20-4.15 (s, 2H), 3.94-3.84 (q,  $J = 7.2$ , 1H), 1.35-1.27 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $\text{C}_{11}\text{H}_{16}\text{ClN}_4\text{O}_2$  271.1 ( $\text{MH}^+$ ). Found  $m/z$  271.0.

## EXAMPLE 32

1-(2S-2-Aminopropionyl)-4-(2-furfuryl)semicarbazide

- 5 Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for furfurylamine. The title compound was obtained as a white solid. Yield: 6 mg (10%); HPLC (15 min. gradient):  $R_t$  = 1.80 min. (>99%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 7.44-7.37 (m, 1H), 6.39-6.34 (m, 1H), 6.26-6.22 (m, 1H), 4.30-4.26 (s, 2H), 4.16-4.05 (q,  $J$  = 7.2, 1H), 1.55-1.47 (d,  $J$  = 7.2, 3H); ES-MS:
- 10 mass calcd for  $\text{C}_9\text{H}_{15}\text{N}_4\text{O}_3$  227.1 ( $\text{MH}^+$ ). Found  $m/z$  227.0.

## EXAMPLE 33

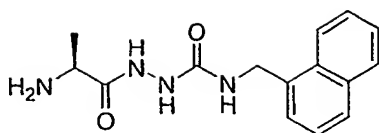
1-(2S-2-Aminopropionyl)-4-[2-(3-indolyl)ethyl]semicarbazide

- 15 Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for tryptamine. The following changes were applied: CDI (22 mg, 0.14 mmol),  $N^t$ -Boc-L-alaninehydrazide (45 mg, 0.22 mmol) in 0.5 mL dry THF, tryptamine (22 mg, 0.14 mmol) in 100  $\mu\text{L}$  DMF. The title compound was obtained as a white solid. Yield: 13 mg (17%); HPLC:  $R_t$  = 3.11 min. (>99%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250
- 20 MHz)  $\delta$  (rotamerism observed) 7.68-7.61 (m, 1H), 7.48-7.42 (m, 1H), 7.27-7.06 (m, 3H), 4.09-3.97 (q,  $J$  = 7.2, 1H), 3.46-3.37 (td,  $J$  = 6.9, 3.14, 2H), 2.95-2.87 (t,  $J$  = 6.9, 2H), 1.48-1.41 (d,  $J$  = 7.2, 3H); ES-MS: mass calcd for  $\text{C}_{14}\text{H}_{20}\text{N}_5\text{O}_2$  290.2 ( $\text{MH}^+$ ). Found  $m/z$  290.2.

## 25 EXAMPLE 34

1-(2S-2-Aminopropionyl)-4-(1-naphthylmethyl)semicarbazide

47

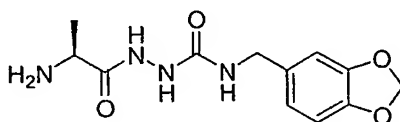


Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for 1-naphthylmethylamine. The title compound was obtained as a white solid. Yield: 42 mg (55%); HPLC:  $R_t$  = 3.50 min. (>97%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 8.03-7.81 (m, 3H), 7.63-7.40 (m, 4H), 4.79-4.74 (s, 2H), 4.13-4.03 (q,  $J$  = 7.2, 1H), 1.52-1.45 (d,  $J$  = 7.2, 3H); ES-MS: mass calcd for  $\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_2$  287.1 ( $\text{MH}^+$ ). Found  $m/z$  287.0.

## EXAMPLE 35

1-(2S-2-Aminopropionyl)-4-piperonylsemicarbazide

10

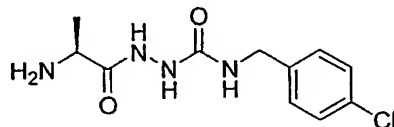


Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for piperonylamine. The title compound was obtained as a white solid. Yield: 14 mg (19%); HPLC:  $R_t$  = 2.70 min. (>95%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 6.85-6.72 (m, 3H), 5.91-5.88 (s, 2H), 4.23-4.20 (s, 2H), 4.16-4.05 (q,  $J$  = 7.2, 1H), 1.55-1.48 (d,  $J$  = 7.2, 3H); ES-MS: mass calcd for  $\text{C}_{12}\text{H}_{17}\text{N}_4\text{O}_4$  281.1 ( $\text{MH}^+$ ). Found  $m/z$  280.9.

## EXAMPLE 36

1-(2S-2-Aminopropionyl)-4-(*p*-chlorobenzyl)semicarbazide

20



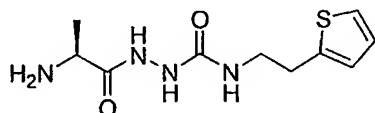
Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for *p*-chlorobenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol),  $N^t$ -Boc-L-alaninehydrazide (90 mg, 0.25 mmol), 4-chlorobenzylamine (35  $\mu\text{L}$ , 0.44 mmol). The title compound was obtained as a white solid. Yield: 52 mg (71%); HPLC:  $R_t$  = 3.30 min. (>97%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 7.37-7.30 (m, 2H), 7.27-7.19 (m, 2H), 4.31-4.26 (s, 2H), 4.16-

4.05 (q,  $J = 7.2$ , 1H), 1.55-1.49 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $C_{11}H_{16}ClN_4O_2$  271.1 ( $MH^+$ ). Found  $m/z$  271.1.

## EXAMPLE 37

1-(2S-2-Aminopropionyl)-4-[2-(thiophen-2-yl)ethyl]semicarbazide

5

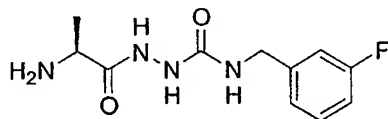


Scheme 2: Following the general procedure of Example 27, except substituting 2-phenylethylamine for 2-(thiophen-2-yl)ethylamine. The following changes were applied: CDI (44 mg, 0.27 mmol),  $N^t$ -Boc-L-alaninehydrazide (90 mg, 0.44 mmol), 2-

10 thiopheneethylamine (35  $\mu$ L, 0.27 mmol). The title compound was obtained as a white solid. Yield: 47 mg (67%); HPLC:  $R_t = 2.72$  min. (>91%);  $^1H$ -NMR ( $D_2O$ , 250 MHz)  $\delta$  (rotamerism observed) 7.28-7.22 (m, 1H), 7.00-6.94 (m, 1H), 6.90-6.86 (m, 1H), 4.12-4.02 (q,  $J = 7.2$ , 1H), 3.45-3.32 (td,  $J = 6.5$ , 3.5, 2H), 3.03-2.93 (t,  $J = 6.6$ , 2H), 1.52-1.47 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $C_{10}H_{17}N_4O_2S$  257.1 ( $MH^+$ ). Found  $m/z$

15 257.2.

## EXAMPLE 38

1-(2S-2-Aminopropionyl)-4-(*m*-fluorobenzyl)semicarbazide

20

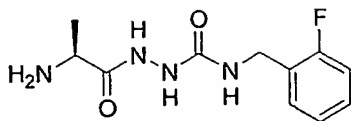
Scheme 2: Following the general procedure of example 27, except substituting 2-phenylethylamine for 3-fluorobenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol, 1.1 eq.),  $N^t$ -Boc-L-alanine (50 mg, 0.25 mmol, 1.0 eq.), 3-fluorobenzylamine (31  $\mu$ L, 0.27 mmol, 1.1 eq.). Yield: 34 mg (55%); HPLC:  $R_t = 2.71$

25 min. (>99%);  $^1H$ -NMR ( $D_2O$ , 250 MHz)  $\delta$  (rotamerism observed) 7.42-7.27 (m, 1H), 7.11-6.94 (m, 3H), 4.34-4.29 (s, 2H), 4.17-4.01 (m, 1H), 1.61-1.53 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $C_{11}H_{15}FN_4O_2$  255.1 ( $MH^+$ ). Found  $m/z$  255.1.

## EXAMPLE 39

1-(2S-2-Aminopropionyl)-4-(*o*-fluorobenzyl)semicarbazide

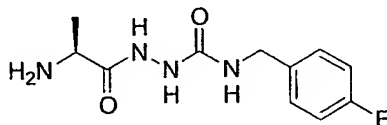
30



Scheme 2: Following the general procedure of example 27, except substituting 2-phenylethylamine for 2-fluorobenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol, 1.1 eq.), *N*<sup>α</sup>-Boc-L-alanine (50 mg, 0.25 mmol, 1.0 eq.), 2-fluorobenzylamine (31 μL, 0.27 mmol, 1.1 eq.). Yield: 27 mg (44%); HPLC: *R*<sub>t</sub> = 2.59 min. (>99%); <sup>1</sup>H-NMR (D<sub>2</sub>O, 250 MHz) δ (rotamerism observed) 7.38-7.28 (m, 2H), 7.22-7.07 (m, 2H), 4.41-4.37 (s, 2H), 4.21-4.08 (m, 1H), 1.61-1.53 (d, *J* = 7.2, 3H); ES-MS: mass calcd for C<sub>11</sub>H<sub>15</sub>FN<sub>4</sub>O<sub>2</sub> 255.1 (MH<sup>+</sup>). Found *m/z* 255.1.

#### 10 EXAMPLE 40

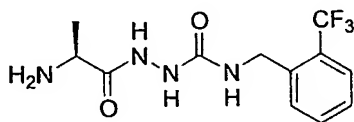
##### 1-(2S-2-Aminopropionyl)-4-(p-fluorobenzyl)semicarbazide



Scheme 2: Following the general procedure of example 27, except substituting 2-phenylethylamine for 4-fluorobenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol, 1.1 eq.), *N*<sup>α</sup>-Boc-L-alanine (50 mg, 0.25 mmol, 1.0 eq.), 4-fluorobenzylamine (31 μL, 0.27 mmol, 1.1 eq.). Yield: 39 mg (62%); HPLC: *R*<sub>t</sub> = 2.70 min. (>99%); <sup>1</sup>H-NMR (D<sub>2</sub>O, 250 MHz) δ (rotamerism observed) 7.39-7.31 (m, 2H), 7.22-7.10 (m, 2H), 4.40-4.33 (s, 2H), 4.28-4.15 (m, 1H), 1.61-1.53 (d, *J* = 7.2, 3H); ES-MS: mass calcd for C<sub>11</sub>H<sub>15</sub>FN<sub>4</sub>O<sub>2</sub> 255.1 (MH<sup>+</sup>). Found *m/z* 255.1.

#### EXAMPLE 41

##### 1-(2S-2-Aminopropionyl)-4-(o-trifluoromethylbenzyl)semicarbazide

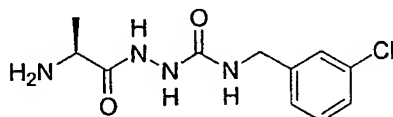


25

Scheme 2: Following the general procedure of example 27, except substituting 2-phenylethylamine for 2-trifluoromethylbenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol, 1.1 eq.), *N*<sup>α</sup>-Boc-L-alanine (50 mg, 0.25 mmol, 1.0 eq.), 2-trifluoromethylbenzylamine (38 μL, 0.27 mmol, 1.1 eq.). Yield: 31 mg (41%);

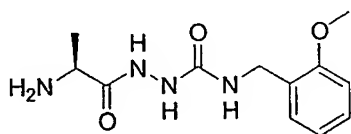
HPLC:  $R_t = 3.37$  min. (>98%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 7.85-7.46 (m, 4H), 4.54-4.52 (s, 2H), 4.22-4.08 (q,  $J = 7.2$ , 1H), 1.61-1.53 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $\text{C}_{12}\text{H}_{15}\text{F}_3\text{N}_4\text{O}_2$  305.1 ( $\text{MH}^+$ ). Found  $m/z$  305.1.

## 5 EXAMPLE 42

1-(2S-2-Aminopropionyl)-4-(*m*-chlorobenzyl)semicarbazide

Scheme 2: Following the general procedure of example 27, except substituting 2-phenylethylamine for 3-chlorobenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol, 1.1 eq.),  $N^{\alpha}$ -Boc-L-alanine (50 mg, 0.25 mmol, 1.0 eq.), 3-chlorobenzylamine (34  $\mu\text{L}$ , 0.27 mmol, 1.1 eq.). Yield: 47 mg (70%); HPLC:  $R_t = 3.17$  min. (>98%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 7.31-7.21 (m, 3H), 7.18-7.11 (m, 1H), 4.31-4.26 (s, 2H), 4.16-4.05 (m,  $J = 7.2$ , 1H), 1.61-1.53 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $\text{C}_{11}\text{H}_{15}\text{ClN}_4\text{O}_2$  271.1 ( $\text{MH}^+$ ). Found  $m/z$  271.1.

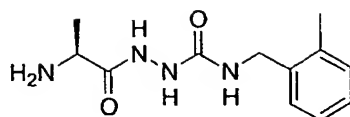
## EXAMPLE 43

1-(2S-2-Aminopropionyl)-4-(*o*-methoxybenzyl)semicarbazide

Scheme 2: Following the general procedure of example 27, except substituting 2-phenylethylamine for 2-methoxybenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol, 1.1 eq.),  $N^{\alpha}$ -Boc-L-alanine (50 mg, 0.25 mmol, 1.0 eq.), 2-methoxybenzylamine (36  $\mu\text{L}$ , 0.27 mmol, 1.1 eq.). Yield: 35 mg (53%); HPLC:  $R_t = 2.91$  min. (>97%);  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ , 250 MHz)  $\delta$  (rotamerism observed) 7.33-7.21 (m, 2H), 7.09-6.99 (m, 2H), 4.31-4.28 (s, 2H), 4.16-4.05 (m, 1H), 1.61-1.53 (d,  $J = 7.2$ , 3H); ES-MS: mass calcd for  $\text{C}_{12}\text{H}_{18}\text{N}_4\text{O}_3$  267.1 ( $\text{MH}^+$ ). Found  $m/z$  267.1.

## EXAMPLE 44

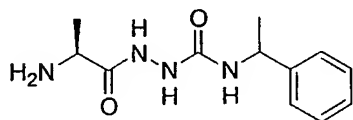
30 1-(2S-2-Aminopropionyl)-4-(*o*-methylbenzyl)semicarbazide



Scheme 2: Following the general procedure of example 27, except substituting 2-phenylethylamine for 2-methylbenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol, 1.1 eq.), *N*<sup>ε</sup>-Boc-L-alanine (50 mg, 0.25 mmol, 1.0 eq.), 2-methylbenzylamine (34  $\mu$ L, 0.27 mmol, 1.1 eq.). Yield: 41 mg (66%); HPLC:  $R_t$  = 2.98 min. (>98%); <sup>1</sup>H-NMR (D<sub>2</sub>O, 250 MHz)  $\delta$  (rotamerism observed) 7.33-7.25 (s, 4H), 4.31-4.26 (s, 2H), 4.25-4.14 (m, 1H), 2.35-2.29 (s, 3H), 1.61-1.53 (d,  $J$  = 7.2, 3H); ES-MS: mass calcd for C<sub>12</sub>H<sub>18</sub>N<sub>4</sub>O<sub>2</sub> 251.1 (MH<sup>+</sup>). Found  $m/z$  251.2.

#### 10 EXAMPLE 45

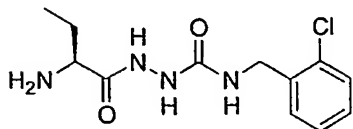
##### 1-(2S-2-Aminopropionyl)-4-(alpha-methylbenzyl)semicarbazide



Scheme 2: Following the general procedure of example 27, except substituting 2-phenylethylamine for (+/-)-alpha-methylbenzylamine. The following changes were applied: CDI (44 mg, 0.27 mmol, 1.1 eq.), *N*<sup>ε</sup>-Boc-L-alanine (50 mg, 0.25 mmol, 1.0 eq.), (+/-)-alpha-methylbenzylamine (35  $\mu$ L, 0.27 mmol, 1.1 eq.). Yield: 20 mg (33%); HPLC:  $R_t$  = 2.91 min. (>92%); <sup>1</sup>H-NMR (D<sub>2</sub>O, 250 MHz)  $\delta$  (rotamerism observed) 7.51-7.31 (m, 5H), 4.90-4.80 (m, 1H), 4.25-4.14 (m, 1H), 1.61-1.55 (d,  $J$  = 7.2, 3H), 1.51-1.45 (d,  $J$  = 6.91, 3H); ES-MS: mass calcd for C<sub>12</sub>H<sub>18</sub>N<sub>4</sub>O<sub>2</sub> 251.1 (MH<sup>+</sup>). Found  $m/z$  251.2.

#### EXAMPLE 46

##### 1-(2S-2-Aminobutanoyl)-4-(o-chlorobenzyl)semicarbazide



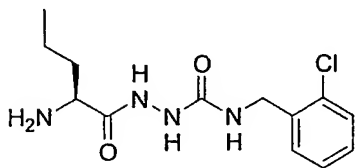
25

Scheme 2 without the isolation of compound II: CDI (45 mg, 0.28 mmol, 1.1 eq.) was added slowly to a solution of *N*<sup>ε</sup>-Boc-L-aminobutyric acid (51 mg, 0.25 mmol, 1.0 eq.) in 1.0 mL dry THF. After addition the solution was stirred for 2 h. Then, hydrazine monohydrate (13.4  $\mu$ L, 0.28 mmol, 1.1 eq.) was added and the resulting solution was



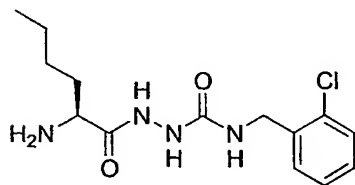
stirred overnight. CDI (45 mg, 0.28 mmol, 1.1 eq.) was added and the solution was stirred for 6 h. 2-Chlorobenzylamine (27  $\mu$ L, 0.23 mmol, 0.9 eq.) was added and the reaction mixture was stirred overnight, and concentrated. The residue was treated with 1 mL 95% TFA/H<sub>2</sub>O for 2 h, and concentrated followed by purification on preparative  
 5 HPLC. Yield: 9.4 mg (13%); HPLC:  $R_t$  = 3.39 min. (>99%); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz)  $\delta$  (rotamerism observed) 7.41-7.28 (m, 2H), 7.26-7.13 (m, 2H), 4.44-4.37 (s, 2H), 3.80-3.71 (t,  $J$  = 6.59, 1H), 1.96-1.79 (m, 2H), 1.07-0.95 (t,  $J$  = 7.54, 3H); ES-MS: mass calcd for C<sub>12</sub>H<sub>17</sub>ClN<sub>4</sub>O<sub>2</sub> 285.1 (MH<sup>+</sup>). Found  $m/z$  285.1.

## EXAMPLE 47

10 1-(2S-2-Aminovaleryl)- 4-(o-chlorobenzyl)semicarbazide

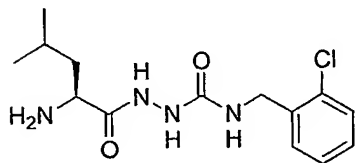
Scheme 2: Following the general procedure of example 46, except substituting *N*<sup>ε</sup>-Boc-L-aminobutyric acid for *N*<sup>ε</sup>-Boc-L-aminovaleric acid. Yield: 11.7 mg (16%); HPLC:  $R_t$  =  
 15 3.36 min. (>99%); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz)  $\delta$  (rotamerism observed) 7.41-7.28 (m, 2H), 7.26-7.13 (m, 2H), 4.44-4.37 (s, 2H), 3.80-3.71 (t,  $J$  = 6.59, 1H), 1.93-1.66 (m, 2H), 1.49-1.34 (m, 2H), 0.98-0.86 (t,  $J$  = 7.22, 3H); ES-MS: mass calcd for C<sub>13</sub>H<sub>19</sub>ClN<sub>4</sub>O<sub>2</sub> 299.1 (MH<sup>+</sup>). Found  $m/z$  299.1.

## EXAMPLE 48

20 1-(2S-2-Aminohexanoyl)- 4-(o-chlorobenzyl)semicarbazide

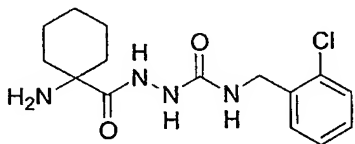
Scheme 2: Following the general procedure of example 46, except substituting *N*<sup>ε</sup>-Boc-L-aminobutyric acid for *N*<sup>ε</sup>-Boc-L-aminocaproic acid. Yield: 30 mg (38%); HPLC:  $R_t$  =  
 25 3.46 min. (>99%); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz)  $\delta$  (rotamerism observed) 7.41-7.28 (m, 2H), 7.26-7.13 (m, 2H), 4.44-4.37 (s, 2H), 3.80-3.71 (t,  $J$  = 6.59, 1H), 1.89-1.68 (m, 2H), 1.43-1.21 (m, 4H), 0.94-0.82 (t,  $J$  = 7.22, 3H); ES-MS: mass calcd for C<sub>14</sub>H<sub>21</sub>ClN<sub>4</sub>O<sub>2</sub> 313.1 (MH<sup>+</sup>). Found  $m/z$  313.1.

## EXAMPLE 49

1-(2S-2-Aminoisocaproyl)- 4-(o-chlorobenzyl)semicarbazide

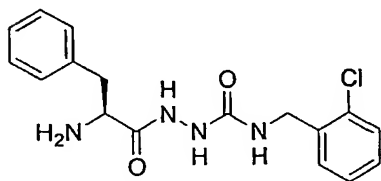
- 5 Scheme 2: Following the general procedure of example 46, except substituting *N*<sup>ε</sup>-Boc-L-aminobutyric acid for *N*<sup>ε</sup>-Boc-L-aminoisocaproic acid. Yield: 17.8 mg (23%); HPLC:  $R_t = 3.45$  min. (>99%); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz)  $\delta$  (rotamerism observed) 7.41-7.28 (m, 2H), 7.26-7.13 (m, 2H), 4.44-4.37 (s, 2H), 3.80-3.71 (t,  $J = 6.59$ , 1H), 1.77-1.57 (m, 3H), 0.98-0.90 (m, 6H); ES-MS: mass calcd for C<sub>14</sub>H<sub>21</sub>ClN<sub>4</sub>O<sub>2</sub> 313.1 (MH<sup>+</sup>). Found  $m/z$
- 10 313.1.

## EXAMPLE 50

1-(1-Aminocyclohexane-1-carbonyl)- 4-(o-chlorobenzyl)semicarbazide

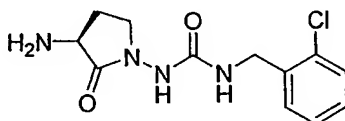
- 15 Scheme 2: Following the general procedure of example 46, except substituting *N*<sup>ε</sup>-Boc-L-aminobutyric acid for Boc-1-amino-1-cyclohexanoic acid. Yield: 3.8 mg (5%); HPLC:  $R_t = 3.42$  min. (>98%); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz)  $\delta$  (rotamerism observed) 7.41-7.28 (m, 2H), 7.26-7.13 (m, 2H), 4.44-4.37 (s, 2H), 2.19-1.20 (m, 10H); ES-MS: mass calcd for C<sub>15</sub>H<sub>21</sub>ClN<sub>4</sub>O<sub>2</sub> 325.1 (MH<sup>+</sup>). Found  $m/z$  325.1.
- 20

## EXAMPLE 51

1-(2S-2-Amino-3-phenyl-propionyl)- 4-(o-chlorobenzyl)semicarbazide

Scheme 2: Following the general procedure of example 46, except substituting *N*<sup>α</sup>-Boc-L-aminobutyric acid for *N*<sup>α</sup>-Boc-L-phenylalanine. Yield: 15.6 mg (18%); HPLC: *R*<sub>t</sub> = 3.71 min. (>97%); <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz) δ (rotamerism observed) 7.38-7.14 (m, 9H), 4.44-4.37 (s, 2H), 3.80-3.61 (m, 3H); ES-MS: mass calcd for C<sub>17</sub>H<sub>19</sub>ClN<sub>4</sub>O<sub>2</sub> 347.1 (MH<sup>+</sup>). Found *m/z* 347.1.

## EXAMPLE 52

1,1-(2*S*-1,4-cyclo-Aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide

Scheme 3:

Step a) Preparation of Cbz-L-Met-NHNHBoc:

Cbz-L-methionine (5 g, 17.65 mmol) was dissolved in dry THF (20 mL) and cooled in an ice-bath. CDI (3.15 g, 19.42 mmol) was added in one portion, resulting in vigorous evolution of gas. After 20 min. BocNHNH<sub>2</sub> (2.57 g, 19.42 mmol) was added and the resulting solution was stirred for a period of 20 h, at which time it was poured into water (150 mL) and extracted with EA (x3). The combined organics were washed once with sat. aq. NaHCO<sub>3</sub>, 10% aq. citric acid and brine. Drying over MgSO<sub>4</sub> followed by evaporation gave an oil which was dried *in vacuo* to give a white solid. Yield: 6.75 g (96%); HPLC: *R*<sub>t</sub> = 5.00 (>99%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 250 MHz) δ 9.00 (br, 1H), 7.29 (m, 5H), 7.27 (br, 1H), 6.15-6.11 (d, *J* = 8.4, 1H), 5.12-4.98 (m, 2H), 4.45-4.40 (m, 1H), 2.57-2.52 (t, *J* = 7.1, 2H), 2.13-1.89 (m, 5H), 1.42 (s, 9H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 250 MHz) δ 171.7, 156.7, 155.7, 136.5, 128.9, 128.5, 128.4, 81.9, 67.5, 52.8, 32.3, 30.3, 28.5, 15.6; ES-MS: mass calcd for C<sub>18</sub>H<sub>28</sub>N<sub>3</sub>O<sub>5</sub>S 398.2 (MH<sup>+</sup>). Found *m/z* 398.1.

Step b) Preparation of Cbz-L-Met-NHNHBoc methyl sulfonium iodide:

Cbz-L-Met-NHNHBoc (6.65 g, 16.73 mmol) was stirred in methyl iodide (25 mL) at rt for a period of 72 h. DCM (50 mL) was added and the yellow solution was concentrated to give a yellow oil, which was dried *in vacuo* to give the sulfonium salt as a yellow solid. Yield: 9.1 g (100%); HPLC: *R*<sub>t</sub> = 3.86 (>99%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 250 MHz) δ 9.33 (br, 1H), 7.27-7.19 (m, 5H), 6.93 (br, 1H), 6.38-6.36 (d, *J* = 7.1, 1H), 5.01 (s, 2H), 4.61-4.59 (m, 1H), 3.75-3.58 (m, 2H), 3.08 (s, 3H), 2.99 (s, 3H), 2.45-2.30 (m, 2H), 1.34 (s, 9H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 250 MHz) δ 171.1, 156.9, 155.8, 136.5, 129.0, 128.6, 128.4, 82.0,

67.7, 52.3, 40.3, 28.7, 26.8, 26.4; ES-MS: mass calcd for  $C_{19}H_{30}N_3O_5S$  412.2 ( $M^+$ ).  
Found  $m/z$  412.1.

Step c) Preparation of 3(S)-benzyloxycarbonylamino-1-tert-

5 butoxycarbonylaminopyrrolidin-2-one:

Cbz-L-Met-NHNHBoc methyl sulfonium iodide (9 g, 16.68 mmol) was dissolved in dry DCM:DMF 1:1 (338 mL). The resulting yellow solution was cooled in an ice-bath under Ar, and NaH (60 % suspension in mineral oil; 1344 mg, 33.6 mmol) was added in one portion. The solution was stirred at 0 °C for 3 h, during which time the color changed  
10 from yellow to various shades of red. The reaction was quenched by addition of EA (115 mL) and water (27 mL), and then stirred overnight at room temperature. The volume was concentrated to ca. 100 mL, diluted with water (300 mL) and extracted with EA (x3). The combined organics were washed with brine, dried over  $MgSO_4$  and concentrated to give the crude product as a semi-solid residue (6 g after drying). This  
15 was dissolved in hot EA:DE 1:2 (60 mL), then cooled slowly to rt and finally to -18 °C. After 1 h a further 10 mL DE and 20 mL n-heptane were added portionwise to the cold solution. Upon storage at -18 °C o. n. a white solid was isolated, washed with n-heptane (x3) and dried. Yield: 2.9 g (50%); HPLC:  $R_t = 4.68$  (>86%)/4.97 (>12%);  
isobaric peaks;  $^1H$ -NMR ( $CDCl_3$ , 250 MHz)  $\delta$  7.28-7.19 (m, 5H), 6.81 (s, 1H), 5.51-5.49  
20 (d,  $J = 7.1$ , 1H), 5.03 (s, 2H), 4.30-4.20 (m, 1H), 3.58-3.37 (m, 2H), 2.58-2.47 (m, 1H),  
2.02-1.70 (m, 1H), 1.39 (s, 9H);  $^{13}C$ -NMR ( $CDCl_3$ , 250 MHz)  $\delta$  172.1, 156.8, 154.7,  
136.7, 128.9, 128.5, 128.4, 82.2, 67.3, 51.2, 46.3, 28.5, 26.4; ES-MS: mass calcd for  
 $C_{17}H_{24}N_3O_5$  350.2 ( $MH^+$ ). Found  $m/z$  350.1.

25 Step d) Preparation of 3(S)-benzyloxycarbonylamino-1-aminopyrrolidin-2-one:

3(S)-benzyloxycarbonylamino-1-tert-butoxycarbonylaminopyrrolidin-2-one (100 mg, 0.286 mmol) was dissolved in HCl in dioxane (4M, 2 mL) and left at rt for 18 h. The solution was evaporated to dryness and the crude residue was purified by preparative HPLC to give a white solid. Yield: 35 mg (49%); HPLC:  $R_t = 3.61$  (>99%); ES-MS:  
30 mass calcd for  $C_{12}H_{16}N_3O_3$  250.1 ( $MH^+$ ). Found  $m/z$  250.1.

Step e) Preparation of 1,1-(2S-1,4-cyclo-benzyloxycarbonylaminobutanoyl)-4-(o-chlorobenzyl)semicarbazide:

3(S)-Benzyloxycarbonylamino-1-aminopyrrolidin-2-one (35 mg, 0.14 mmol) and NEM  
35 (35  $\mu$ L, 0.28 mmol) were dissolved in dry THF (1.5 mL), and CDI (25 mg, 0.154 mmol) was added. After a period of 21 h at rt o-chlorobenzylamine (19  $\mu$ L, 0.154 mmol) was

added. After 4.5 h the crude product was purified by preparative HPLC to give a white solid. Yield: 20 mg (34%); HPLC:  $R_t = 4.84$  (>99%); ES-MS: mass calcd for  $C_{20}H_{22}N_4O_4$  417.1 ( $MH^+$ ). Found  $m/z$  417.0.

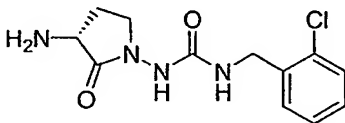
5 Step f) Preparation of 1,1-(2S-1,4-cyclo-aminobutanoyl)-4-(o-chlorobenzyl)semicarbazide:

1,1-(2S-1,4-cyclo-benzoyloxycarbonylaminobutanoyl)-4-(o-chlorobenzyl)semicarbazide (19 mg, 46  $\mu$ mol) was dissolved in glacial acetic acid (300  $\mu$ L). HBr in acetic acid (5.7 M, 20  $\mu$ L, 114  $\mu$ mol) was added. After 22 h a further 20  $\mu$ L HBr in acetic acid was  
10 added, and yet another 20  $\mu$ L after a further 3.5 h. Three hours later the crude product was purified by preparative HPLC to give a white solid. Yield: 10.7 mg (83%); HPLC:  $R_t = 3.34$  (>99%);  $^1H$ -NMR ( $CDCl_3$ , 250 MHz)  $\delta$  7.56-7.34 (m, 4H), 4.59 (s, 2H), 4.29-4.21 (t,  $J = 9.1$ , 1H), 3.85-3.66 (m, 2H), 2.78-2.66 (m, 1H), 2.34-2.21 (m, 1H); ES-MS: mass calcd for  $C_{12}H_{16}ClN_4O_2$  283.1 ( $MH^+$ ). Found  $m/z$  283.1.

15

EXAMPLE 53

1,1-(2R-1,4-cyclo-Aminobutanoyl)-4-(o-chlorobenzyl)semicarbazide



20 Scheme 3:

Step a) Preparation of Cbz-D-Met-NHNHBoc:

Starting from Cbz-D-methionine (2 g, 7.06 mmol), following the procedure for Example 52 a) gave a white solid. Yield: 2.7 g (71%); HPLC:  $R_t = 4.03$  (shorter gradient; >99%);  $R_f$  (PE:EA 1:1) = 0.62;  $^1H$ -NMR ( $CDCl_3$ , 250 MHz)  $\delta$  8.92 (br, 1H), 7.32 (m, 5H), 7.09  
25 (br, 1H), 6.09-6.05 (d,  $J = 8.2$ , 1H), 5.15-5.00 (m, 2H), 4.54-4.45 (m, 1H), 2.60-2.55 (t,  $J = 7.1$ , 2H), 2.19-1.89 (m, 5H), 1.45 (s, 9H);  $^{13}C$ -NMR ( $CDCl_3$ , 250 MHz)  $\delta$  173.2, 156.8, 155.8, 136.4, 128.9, 128.6, 128.5, 82.2, 67.6, 52.8, 32.2, 30.3, 28.5, 15.6; ES-MS: mass calcd for  $C_{18}H_{28}N_3O_5S$  398.2 ( $MH^+$ ). Found  $m/z$  398.2.

30 Step b) Preparation of Cbz-D-Met-NHNHBoc methyl sulfonium iodide:

Starting from Cbz-D-Met-NHNHBoc (2.6 g, 6.55 mmol), following the procedure for Example 52 b) gave a yellow solid. Yield: 3.5 g (100%); HPLC:  $R_t = 3.71$  (>99%);  $^1H$ -NMR ( $CDCl_3$ , 250 MHz)  $\delta$  9.55 (br, 1H), 7.58 (br, 1H), 7.28-7.19 (m, 5H), 6.73-6.70 (d,  $J = 7.5$ , 1H), 5.00 (s, 2H), 4.50-4.47 (m, 1H), 3.68-3.59 (m, 2H), 3.08 (s, 3H), 3.03 (s,

3H), 2.33-2.21 (m, 2H), 1.35 (s, 9H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 250 MHz)  $\delta$  171.8, 156.9, 155.8, 136.5, 128.8, 128.5, 128.3, 81.4, 67.3, 52.0, 40.0, 28.6, 26.3, 26.0; ES-MS: mass calcd for  $\text{C}_{19}\text{H}_{30}\text{N}_3\text{O}_5\text{S}$  412.2 ( $\text{M}^+$ ). Found  $m/z$  412.2.

5 Step c) Preparation of 3(R)-benzyloxycarbonylamino-1-tert-butoxycarbonylaminopyrrolidin-2-one:

Starting from Cbz-D-Met-NHNHBoc methyl sulfonium iodide (1.7 g, 3.15 mmol), following the procedure for Example 52 c) gave the crude product as a clear oil (1.1 g after drying). This was purified on silica, using EA:n-heptane 2:1 to give a white solid.

10 Yield: 660 mg (60%); HPLC:  $R_t = 4.47$  (>66%)/4.80 (>28%); isobaric peaks;  $R_f$  (PE:EA 1:1) = 0.3;  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 250 MHz)  $\delta$  7.18-7.10 (m, 5H), 6.65 (br, 1H), 5.36 (br, 1H), 4.93 (s, 2H), 4.19-4.06 (m, 1H), 3.44-3.27 (m, 2H), 2.49-2.38 (m, 1H), 1.90-1.77 (m, 1H), 1.29 (s, 9H); ES-MS: mass calcd for  $\text{C}_{17}\text{H}_{24}\text{N}_3\text{O}_5$  350.2 ( $\text{MH}^+$ ). Found  $m/z$  350.1.

15 Step d) Preparation of 3(R)-benzyloxycarbonylamino-1-aminopyrrolidin-2-one:

3(R)-Benzyloxycarbonylamino-1-tert-butoxycarbonylaminopyrrolidin-2-one (650 mg, 1.86 mmol) was dissolved in DCM (10 mL). TFA:H<sub>2</sub>O 95:5 (12 mL) was added and the resulting solution was left at -18 °C for 3 days, at which point it was taken to rt for 6 h and finally concentrated *in vacuo*. The crude, residual oil was purified by preparative

20 HPLC to give a white solid. Yield: 56 mg (12%); HPLC:  $R_t = 3.36$  (>95%); ES-MS: mass calcd for  $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_3$  250.1 ( $\text{MH}^+$ ). Found  $m/z$  250.1.

Step e) Preparation of 1,1-(2R-1,4-cyclo-benzyloxycarbonylaminobutanoyl)-4-(o-chlorobenzyl)semicarbazide:

25 Starting from 3(R)-benzyloxycarbonylamino-1-aminopyrrolidin-2-one (28 mg, 0.113 mmol), following the procedure for Example 52 e) gave a white solid. Yield: 13 mg (28%); HPLC:  $R_t = 4.90$  (>95%); ES-MS: mass calcd for  $\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_4$  417.1 ( $\text{MH}^+$ ). Found  $m/z$  417.1.

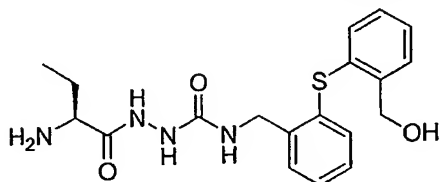
30 Step f) Preparation of 1,1-(2R-1,4-cyclo-aminobutanoyl)-4-(o-chlorobenzyl)semicarbazide:

1,1-(2R-1,4-cyclo-benzyloxycarbonylaminobutanoyl)-4-(o-chlorobenzyl)semicarbazide (13 mg, 31  $\mu\text{mol}$ ) was dissolved in neat TFA (625  $\mu\text{L}$ , 8 mmol). Thioanisole (185  $\mu\text{L}$ , 1.56 mmol) was added, followed by TMSBr (41  $\mu\text{L}$ , 312  $\mu\text{mol}$ ). After 3 h at rt the

35 solution was concentrated, and the crude product was purified by preparative HPLC to give a white solid. Yield: 3 mg (34%); HPLC:  $R_t = 3.15$  (>99%);  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 250

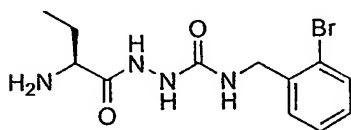
MHz)  $\delta$  7.57-7.35 (m, 4H), 4.60 (s, 2H), 4.30-4.22 (t,  $J = 9.1$ , 1H), 3.86-3.67 (m, 2H), 2.79-2.67 (m, 1H), 2.34-2.18 (m, 1H); ES-MS: mass calcd for  $C_{12}H_{16}ClN_4O_2$  283.1 ( $MH^+$ ). Found  $m/z$  283.1.

## 5 EXAMPLE 54

1-(2S-2-Aminobutanoyl)-4-[o-(o-hydroxymethylphenylthio)benzyl]semicarbazide

- Scheme 2: a) Preparation of  $N^{\alpha}$ -Boc-L-aminobutyric acid hydrazide: CDI (1.76 g, 10.8 mmol) was added slowly to a solution of  $N^{\alpha}$ -Boc-L-aminobutyric acid (2.0 g, 9.84 mmol) in 20 mL dry THF. After addition the solution was stirred for 1 h. Then, hydrazine monohydrate (1.44 mL, 29.5 mmol) was added and the resulting solution was stirred o.n. The reaction mixture was concentrated and the white solid re-dissolved in 1,4-dioxane and lyophilized overnight yielding 3.85 g (180%) of crude product. The solid was a mixture of imidazole and desired product (imidazole:product 2:1, determined by  $^1H$ -NMR) and was used without further purification.  $^1H$ -NMR ( $DMSO-d_6$ , 250 MHz)  $\delta$  9.08-8.89 (s, 1H), 6.75-6.10 (d,  $J = 8.2$ , 1H), 3.79-3.64 (q,  $J = 7.91$ , 14.4, 1H), 1.61-1.34 (m, 2H), 1.34-1.18 (s, 9H), 0.81-0.63 (t,  $J = 7.54$ , 3H)
- b) CDI (41 mg, 0.26 mmol) was added to a solution of  $N^{\alpha}$ -Boc-L-aminobutyric acid hydrazide (50 mg, 0.23 mmol) in 1.0 mL dry THF. The resulting solution was stirred overnight and 2-[2-(aminomethyl)phenylthio]-benzyl alcohol (0.26 mmol) dissolved in 1.0 mL dry THF was added. The reaction mixture was stirred o.n., and then concentrated. The residue was treated with 1 mL 95% TFA/ $H_2O$  for 5 min, and concentrated followed by purification by preparative HPLC. The title compound was obtained as a white solid. Yield: 25 mg (28%); HPLC:  $R_t = 3.66$  min. (>98%); ES-MS: mass calcd for  $C_{19}H_{24}N_4O_2S$  389.2 ( $MH^+$ ). Found  $m/z$  389.1.

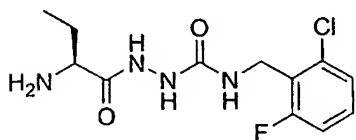
## EXAMPLE 55

1-(2S-2-Aminobutanoyl)-4-(o-bromobenzyl)semicarbazide

Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for *o*-bromobenzylamine. Yield: 49 mg (65%); HPLC:  $R_t = 3.12$  min. (>99%); ES-MS: mass calcd for  $C_{12}H_{17}BrN_4O_2$  329.1 ( $MH^+$ ). Found  $m/z$  329.1

## 5 EXAMPLE 56

1-(2S-2-Aminobutanoyl)-4-(2-chloro-6-fluorobenzyl)semicarbazide

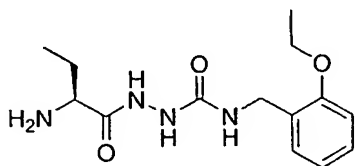


10 Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for 2-chloro-6-fluorobenzylamine. Yield: 14 mg (20%); HPLC:  $R_t = 3.03$  min. (>99%); ES-MS: mass calcd for  $C_{12}H_{17}ClFN_4O_2$  303.1 ( $MH^+$ ). Found  $m/z$  303.0.

## EXAMPLE 57

1-(2S-2-Aminobutanoyl)-4-(*o*-ethoxybenzyl)semicarbazide

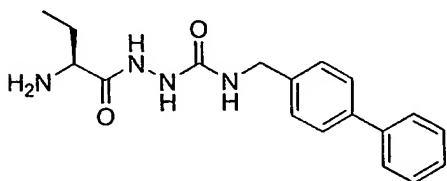
15



20 Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for *o*-ethoxybenzylamine. Yield: 28 mg (41%); HPLC:  $R_t = 3.27$  min. (>99%); ES-MS: mass calcd for  $C_{14}H_{23}N_4O_3$  295.2 ( $MH^+$ ). Found  $m/z$  295.2

## EXAMPLE 58

1-(2S-2-Aminobutanoyl)-4-([1,1'-biphenyl-4-yl]methyl)semicarbazide



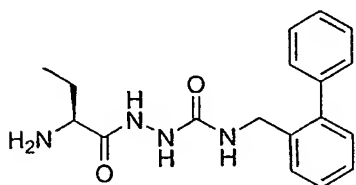
25



Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for (1,1'-biphenyl-4-yl)methylamine. Yield: 34 mg (45%); HPLC:  $R_t = 3.86$  min. (>99%); ES-MS: mass calcd for  $C_{18}H_{23}N_4O_2$  327.2 ( $MH^+$ ). Found  $m/z$  327.2.

## 5 EXAMPLE 59

1-(2S-2-Aminobutanoyl)-4-([1,1'-biphenyl-2-yl]methyl)semicarbazide

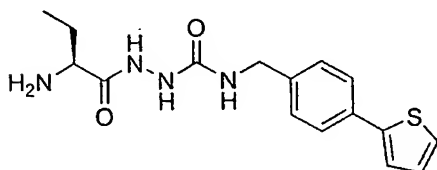


10 Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for (1,1'-biphenyl-2-yl)methylamine. Yield: 63 mg (83%); HPLC:  $R_t = 3.97$  min. (>97%); ES-MS: mass calcd for  $C_{18}H_{23}N_4O_2$  327.2 ( $MH^+$ ). Found  $m/z$  327.4.

## EXAMPLE 60

1-(2S-2-Aminobutanoyl)-4-(p-[thiophen-2-yl]benzyl)semicarbazide

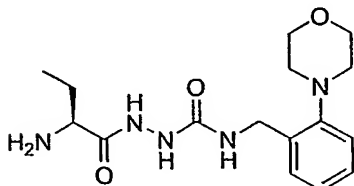
15



20 Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for *p*-(thiophen-2-yl)benzylamine. Yield: 29 mg (37%); HPLC:  $R_t = 3.92$  min. (>99%); ES-MS: mass calcd for  $C_{16}H_{21}N_4O_2S$  333.1 ( $MH^+$ ). Found  $m/z$  333.2.

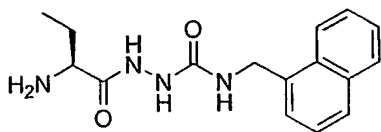
## EXAMPLE 61

1-(2S-2-Aminobutanoyl)-4-(o-[N-morpholinyl]benzyl)semicarbazide



Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for *o*-(*N*-morpholinyl)benzylamine. Yield: 32 mg (41%); HPLC:  $R_t = 2.55$  min. (>99%); ES-MS: mass calcd for  $C_{16}H_{26}N_5O_3$  336.2 ( $MH^+$ ). Found  $m/z$  336.3.

## 5 EXAMPLE 62

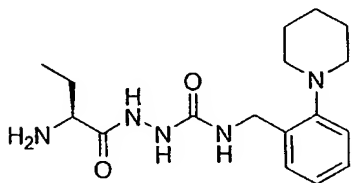
1-(2*S*-2-Aminobutanoyl)-4-(1-naphthylmethyl)semicarbazide

10 Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for 1-naphthylmethylamine. Yield: 32 mg (46%); HPLC:  $R_t = 3.53$  min. (>99%); ES-MS: mass calcd for  $C_{16}H_{21}N_4O_2$  301.2 ( $MH^+$ ). Found  $m/z$  301.3.

## EXAMPLE 63

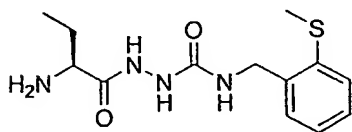
1-(2*S*-2-Aminobutanoyl)-4-(*o*-[*N*-piperidinyl]benzyl)semicarbazide

15



20 Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for *o*-(*N*-piperidinyl)benzylamine. Yield: 7 mg (9%); HPLC:  $R_t = 2.71$  min. (>96%); ES-MS: mass calcd for  $C_{17}H_{28}N_5O_2$  334.2 ( $MH^+$ ). Found  $m/z$  334.2.

## EXAMPLE 64

1-(2*S*-2-Aminobutanoyl)-4-(*o*-methylthiobenzyl)semicarbazide

25

Scheme 2: Following the general procedure of example 54, except substituting 2-[2-(aminomethyl)phenylthio]-benzyl alcohol for o-methylthiobenzylamine. Yield: 20 mg (29%); HPLC:  $R_t = 3.27$  min. (99>%); ES-MS: mass calcd for  $C_{13}H_{21}N_4O_2S$  297.1 (MH<sup>+</sup>). Found  $m/z$  297.2.

5

### Biological Assays

The compounds of this invention may be tested in one of several biological assays to determine their pharmacological properties.

#### 10 Human dipeptidyl peptidase I (DPP-I) assay

Using this assay, the IC<sub>50</sub> value of a compound of the invention as a DPP-I inhibitor was determined using an AFC substrate.

#### *Assay buffer (pH 6.0):*

15 100 mM sodium phosphate (8.9 g Na<sub>2</sub>HPO<sub>4</sub>; M = 177.99), 150 mM KCl (5.6 g KCl; M = 74.6) and 1.5 mM EDTA (279 mg EDTA; M = 372.2) was dissolved in 500 mL H<sub>2</sub>O, and pH was adjusted to 6.0. Cysteine\*HCl (Sigma C-1276; M = 157.6), 1 mg/mL assay buffer, corresponding to 6 mM, was added to the solution for activation of the DPP-I enzyme.

20

#### *Substrate:*

Gly-Phe-AFC\*TFA (Enzyme Systems Products AFC-033) was used as the substrate for determination of IC<sub>50</sub> values. K<sub>m</sub> was 270 μM. The substrate was solubilized in DMSO to give a 7.5 mM stock solution (2.2 mg of substrate was added to 0.5 mL  
25 DMSO).

#### *DPP-I:*

Human DPP-I (hDDP-I; obtained from UniZyme A/S, DK-2970 Hørsholm, Denmark) was stored at -20 °C in a buffer containing 2.5 mM Na-phosphate, 150 mM NaCl, 2  
30 mM cysteamine, 50% glycerol, pH 7.0 at a concentration of 2.5 mg/mL. This stock solution was diluted 200 times in the assay buffer.

#### *Assay conditions:*

The assay was performed in 96-well plates. Assay buffer (230 μL) was added to the  
35 well, followed by 10 μL of diluted DPP-I, corresponding to 9.1 nM in the assay. Then 5 μL of either DMSO (control) or test substance in varying concentrations was added,

and the solution was mixed. The plate was incubated at 37 °C for 10 minutes, followed by addition of 5 µL of 7.5 mM substrate (corresponding to 150 µM in the assay). The excitation wavelength was 400 nm, and the emission was measured at 505 nm for 10 minutes at 37 °C. Each measurement was made in duplicate. In the software (SOFTmax Pro) used for data collection from the fluorometer (Molecular Devices: Gemini XS), it was ensured that the measured slopes were linear ( $R^2 > 0.99$ ). Data were exported to GraphPad Prism and nonlinear regression was performed using the option Sigmoidal dose-response (variable slope).

10 Human liver cathepsin B assay

Using this assay, the selectivity of a compound of the invention for hDPP-I over human cathepsin B was determined, using a fluorogenic substrate.

*Assay buffer (pH 6.1):*

15 0.1 M MES buffer (1.95 g; M = 195.2) and 1 mM EDTA (37 mg; M = 372.2) were mixed in 100 mL H<sub>2</sub>O and pH adjusted to 6.1. DTT (10 µL, 0.5 M) was added for activation of the enzyme (corresponding to 5 mM).

*Cathepsin B:*

20 Human liver cathepsin B (Enzyme System Products, CAT-B; stock 25 µg / 54 µL = 463 ng/µL). One aliquot was diluted to a concentration of 40 ng/µL by adding 53 µL assay buffer (without DTT). Just prior to experiment, two more dilution steps were performed:  
4 ng/µL: 5 µL (40 ng/µL) + 45 µL buffer (without DTT)  
0.1 ng/µL: 5 µL (4 ng/µL) + 285 µL buffer (without DTT)

25

*Substrate:*

Boc-Leu-Arg-Arg-AFC•2TFA (Enzyme System Products AFC113). Stock solution made (20 mM; 15.1 mg dissolved in 1 ml DMSO). Diluted further in H<sub>2</sub>O to 10 mM.  $K_m$  for this substrate has been determined to be 600 µM.

30

*Assay conditions:*

The assay was performed in 96-well plates. 84 µL assay buffer was added to the well followed by 10 µL 1% DMSO in assay buffer (control) or a compound of the invention (10 µM in the assay). Then 10 µL (0.1 ng/µL, corresponding to 1 ng in assay) enzyme was added, and 5 min. later 6 µL substrate (10 mM, corresponding to 600 µM in the assay) was added. The excitation wavelength was 400 nm, and the emission was

35

measured at 505 nm for 10-20 minutes at 37 °C. Each measurement was made in duplicate. In the software (SOFTmax Pro) used for data collection from the fluorometer (Molecular Devices: Gemini XS), it was ensured that the measured slopes were linear ( $R^2 > 0.99$ ). Data were exported to GraphPad Prism and nonlinear regression was performed using the option Sigmoidal dose-response (variable slope).

#### Human cathepsin G assay

Using this assay, the selectivity of a compound of the invention for hDPP-I over human cathepsin G was determined, using a chromogenic substrate.

#### Assay buffer (pH 8.3):

Buffer containing 100 mM Tris/HCl pH 8.3 (25 °C), 500 mM NaCl, and 20 mM CaCl<sub>2</sub> prepared. DTNB (5,5'-dithio-bis(2-nitrobenzoic acid)) was added just prior to experiment (10 µL 1.25 mM/well).

#### Cathepsin G:

Human cathepsin G (Calbiochem Cat # 219373, 50 ng/µL = 0.1 mU/µL stock) was reconstituted in 50 mM sodium acetate and 150 mM NaCl (pH 5.5), and stored at -20 °C. Diluted to 2 ng/µL in buffer just prior to experiment.

#### Substrate:

Suc-Ala-Ala-Pro-PheI-SBzl (Bachem Cat # 4009924.0050) diluted to 1 mM in buffer from 10 mM stock in buffer.  $K_m$  determined to 100-200 µM.

#### Assay conditions:

The assay was performed in 96-well plates. 60 µL assay buffer was added to the well followed by 10 µL DTNB (1.25 mM), and 10 µL of a compound of the invention (100 µM, diluted with assay buffer to give 10 µM in the assay). Then 10 µL (2 ng/µL) enzyme was added, and 10 min. later (at 37 °C) 10 µL substrate (100 µM final concentration) was added. The absorption was measured at 410 nm for 10 minutes at 37 °C. Each measurement was made in duplicate. In the software (SOFTmax Pro) used for data collection from the UV-spectrometer it was ensured that the measured slopes were linear ( $R^2 > 0.99$ ). Data were exported to GraphPad Prism and nonlinear regression was performed using the option Sigmoidal dose-response (variable slope).

#### Human liver cathepsin H assay

Using this assay, the selectivity of a compound of the invention for hDPP-I over human cathepsin H was determined, using a fluorogenic substrate.

Assay buffer (pH 6.0):

- 5 50 mM sodium phosphate (0.89 g  $\text{Na}_2\text{HPO}_4$ ; M = 177.99), 2.0 mM EDTA (74 mg EDTA; M = 372.2), 0.012 % Triton-X (390  $\mu\text{L}$  3 %) dissolved in 100 mL  $\text{H}_2\text{O}$  and pH adjusted to 6.0.

*Cathepsin H:*

- 10 Human liver cathepsin H (Enzyme System Products, Cath-1; 25  $\mu\text{g}$ ) was solubilized in 60  $\mu\text{L}$  enzyme buffer (giving a stock with a concentration of 417 ng/ $\mu\text{L}$ ). Enzyme stock (5  $\mu\text{L}$ ) was diluted with 1245  $\mu\text{L}$  enzyme buffer. Prior to running experiment 1  $\mu\text{L}$  0.5 M DTT/100  $\mu\text{L}$  enzyme solution was added. Incubated for 5 minutes on ice, then added to reaction mixture.

15

*Substrate:*

ARG-AFC\*2HBR (Enzyme System Products AF002; 10.6 mg) was dissolved in 1 mL DMSO, giving a 20 mM solution.  $K_m$  for this substrate has been determined to be 27  $\mu\text{M}$

20

*Assay conditions:*

- The assay was performed in 96-well plates. 50  $\mu\text{L}$  assay buffer was added to the well followed by 25  $\mu\text{L}$  reference inhibitor (cystatin; stock 1 mg/mL, diluted with assay buffer to give 10 nM in assay) or a compound of the invention (diluted with assay buffer to give 10  $\mu\text{M}$  in the assay). Then 25  $\mu\text{L}$  (40 ng) enzyme was added, and 1 min. later (at 25 37 °C), 100  $\mu\text{L}$  ARG-AFC substrate (15  $\mu\text{M}$  in the assay) was added. The excitation wavelength was 400 nm, and the emission was measured at 505 nm for 10-20 minutes at 37 °C. Each measurement was made in duplicate. In the software (SOFTmax Pro) used for data collection from the fluorometer (Molecular Devices: Gemini XS), it was ensured that the measured slopes were linear ( $R^2 > 0.99$ ). Data were exported to GraphPad Prism and nonlinear regression was performed using the option Sigmoidal dose-response (variable slope).

30

Human liver cathepsin L assay

- 35 Using this assay, the selectivity of a compound of the invention for hDPP-I over human cathepsin L was determined, using a fluorogenic substrate.

Assay buffer (pH 5.5):

20 mM sodium acetate (M = 82.04; 164 mg), 4.0 mM EDTA (M = 372.2; 149 mg),

0.012 % Triton-X (390 µL 3 %) dissolved in 100 mL H<sub>2</sub>O and pH adjusted to 5.5.

5

*Cathepsin L:*

Human liver cathepsin L (Enzyme System Products, CatL-1; 5 µL of a 1.61 µU/µL stock)

was solubilized in 2500 µL enzyme buffer. Prior to running experiment, 1 µL 0.5 M

DTT/100 µL enzyme solution was added. Incubated for 5 minutes on ice, then added to

10

reaction mixture.

*Substrate:*

Z-Phe-ARG-AFC\*TFA (Enzyme System Products AF052; 15.6 mg) was dissolved in 1

mL DMSO, giving a 20 mM solution.

15

*Assay conditions:*

The assay was performed in 96-well plates. 50 µL assay buffer was added to the well followed by 25 µL reference inhibitor (cystatin, stock 1 mg/mL, diluted with assay buffer to give 25 nM in assay) or a compound of the invention (diluted with assay buffer to

20

give 10 µM in the assay). Then 25 µL (80 nU) enzyme was added, and 1 min. later (at

37 °C) 100 µL substrate (10 µM in the assay) was added. The excitation wavelength was 400 nm, and the emission was measured at 505 nm for 10-20 minutes at 37°C.

Each measurement was made in duplicate. In the software (SOFTmax Pro) used for data collection from the fluorometer (Molecular Devices: Gemini XS), it was ensured

25

that the measured slopes were linear ( $R^2 > 0.99$ ). Data were exported to GraphPad

Prism and nonlinear regression was performed using the option Sigmoidal dose-response (variable slope).

*Aspergillus* DPP-IV assay

30

Using this assay, the selectivity of a compound of the invention for hDPP-I over

*Aspergillus* DPP-IV was determined, using a fluorogenic substrate.

Assay buffer (pH 7.4):

50 mM Tris, 0.1% Triton.

35

*Aspergillus* DPP-IV:

*Aspergillus* DPP-IV (Enzyme System Products, SPE01; 5 mU dissolved in 25  $\mu$ L assay buffer, giving a stock with a concentration of a 0.2 mU/ $\mu$ L). Prior to running experiment, 5  $\mu$ L of enzyme stock solution was diluted to 0.004 mU/ $\mu$ L with 245  $\mu$ L buffer.

5    *Substrate:*

Gly-Pro-AFC-TFA (Enzyme System Products AF039; 10 mg) was dissolved in 1 mL DMSO, giving a 20 mM solution. Diluted further in buffer to 1 mM.

*Assay conditions:*

- 10    The assay was performed in 96-well plates. 60  $\mu$ L assay buffer was added to the well followed by 10  $\mu$ L 1% DMSO in assay buffer (control) or a compound of the invention (100  $\mu$ M, corresponding to 10  $\mu$ M in the assay). Incubated at 37 °C, then 10  $\mu$ L enzyme (corresponding to 0.04 mU in assay) was added, followed by 10  $\mu$ L substrate (100  $\mu$ M in the assay). The excitation wavelength was 400 nm, and the emission was measured
- 15    at 505 nm for 10-20 minutes at 30 °C. Each measurement was made in duplicate. In the software (SOFTmax Pro) used for data collection from the fluorometer (Molecular Devices: Gemini XS), it was ensured that the measured slopes were linear ( $R^2 > 0.99$ ). Data were exported to GraphPad Prism and nonlinear regression was performed using the option Sigmoidal dose-response (variable slope).

20

Human neutrophil elastase assay

Using this assay, the selectivity of a compound of the invention for hDPP-I over human neutrophil elastase was determined, using a fluorogenic substrate.

25    *Assay buffer (pH 7.5):*

Buffer containing 100 mM Tris/HCl pH 7.5 (25 °C), and 500 mM NaCl.

*Neutrophil elastase:*

- Human neutrophil elastase (Calbiochem Cat # 324681, 50 ng/ $\square$ L stock) was
- 30    reconstituted in 50 mM sodium acetate pH 5.5 and 200 mM NaCl, then stored at -20 °C. Diluted to 1 ng/ $\mu$ L in buffer just prior to experiment.

*Substrate:*

- MeOSuc-Ala-Ala-Pro-Val-AMC (Calbiochem Cat # 324740) was dissolved in buffer to 4
- 35    mM (398  $\mu$ L/mg) just prior to experiment.  $K_m$  was determined to be 560  $\mu$ M.



*Assay conditions:*

The assay was performed in 96-well plates. 80  $\mu$ L assay buffer was added to the well followed by 10  $\mu$ L enzyme (1 ng/ $\mu$ L). Incubated for 10 min. at 37 °C, then 10  $\mu$ L substrate was added, and subsequently a compound of the invention (10  $\mu$ M in the assay) The excitation wavelength was 380 nm, and the emission was measured at 460 nm for 10-20 minutes at 37 °C. Each measurement was made in duplicate. In the software (SOFTmax Pro) used for data collection from the fluorometer (Molecular Devices: Gemini XS), it was ensured that the measured slopes were linear ( $R^2 > 0.99$ ). Data was exported to GraphPad Prism and nonlinear regression was performed using the option sigmoidal dose-response (variable slope).

Tryptase assay

Using this assay, the selectivity of a compound of the invention for hDPP-I over  $\beta$ -tryptase was determined, using a fluorogenic substrate.

Assay buffer (pH 7.5):

0.1 M HEPES buffer (pH 7.5) with 0.5 M NaCl.

*Tryptase:*

Rh-Skin  $\beta$ -tryptase (Promega # G7061) was diluted to a final stock solution of 0.08  $\mu$ g/ $\mu$ L in assay buffer.

Rh-lung  $\beta$ -tryptase (Promega # G5631) was diluted to a final stock solution of 0.08  $\mu$ g/ $\mu$ L in assay buffer.

*Substrate:*

Z- ARG-AMC\*HCl (BACHEM I1130; 10 mg) was dissolved in 1 mL DMSO, giving a 20 mM solution.  $K_m$  was determined to be  $> 400 \mu$ M.

*Assay conditions:*

The assay was performed in 96-well plates. 85  $\mu$ L assay buffer was added to the well followed by 5  $\mu$ L enzyme stock. Then 5  $\mu$ L substrate was added, and subsequently 5  $\mu$ L 4% DMSO in assay buffer (control) or a compound of the invention (10  $\mu$ M in the assay) The excitation wavelength was 380 nm, and the emission was measured at 460 nm for 10-20 minutes at 37°C. Each measurement was made in duplicate. In the software (SOFTmax Pro) used for data collection from the fluorometer (Molecular Devices: Gemini XS), it was ensured that the measured slopes were linear ( $R^2 > 0.99$ ).

Data were exported to GraphPad Prism and nonlinear regression was performed using the option Sigmoidal dose-response (variable slope).

#### Human CYP1A2 assay

- 5 This assay was used to determine the effect of a compound of the invention on the CYP1A2 liver metabolising enzyme.

Vivid® CYP1A2 Blue Screening Kit from PanVera was used.

#### *Kit components:*

- 10 Vivid®CYP450 Reaction Buffer I: 200 mM potassium phosphate buffer, pH 8.  
CYP1A2 baculosomes® reagent: CYP1A2 and NADH-P450 reductase (P450-specific content: 1.1 µM). Microsomes prepared from insect cells that were infected with baculovirus containing the cDNA for human CYP1A2 and rabbit cytochrome P450 reductase.
- 15 Regenerating System: 333 mM glucose-6-phosphate and 40 U/mL glucose-6-phosphate dehydrogenase in 100 mM potassium phosphate buffer, pH 8.  
Vivid® CYP1A2 Blue Substrate.  
Vivid® Blue Standard: 3-cyano-7-hydroxycoumarin.  
10 mM NADP<sup>+</sup> in 100 mM potassium phosphate buffer, pH 8.

20

#### *Procedure*

Tubes thawed on ice. 2x reaction buffer dispensed (room temperature) in two eppendorf tubes (for Mix A and Mix B).

- 25 Controls or compounds of the invention: Diluted to a concentration of 50 µM. Positive control compound:  $\alpha$ -naphthoflavone.  
Negative controls: DMSO and H<sub>2</sub>O.

Mix A: 485 µL reaction buffer, 10 µL regeneration system, 5 µL Baculosome reagent.

- 30 Mixed gently and placed on ice.

Mix B: 88.5 µL reaction buffer, 1.5 µL substrate, 10 µL 10 mM NADP<sup>+</sup>.

- In a 96-well plate (Costar c-3904), 40 µL of 50 µM sample dispensed. 50 µL Mix A added. Incubated 20 min. at rt. Then, 10 µL of Mix B added. Fluorescence determined
- 35

(Molecular Devices, SPECTRAmax Gemini EM): Ex: 409 nm, Em: 460 nm, at 37 °C for 20 min. (Automix: 5 s, top read.).

Following the procedure for CYP1A2, using the appropriate kits below, the effect of a compound of the invention on the other CYP enzymes was determined:

5

Vivid® CYP2C9 Red Screening Kit from PanVera.

Vivid® CYP2C19 Red Screening Kit from PanVera.

Vivid® CYP2D6 Cyan Screening Kit from PanVera.

Vivid® CYP3A4 Red Screening Kit from PanVera.

10

#### Cellular DPP-I assay

The following assay was used to determine the inhibitory effect of compounds of the invention on cellular rat DPP-I.

15

#### *Cell culture:*

The RBL-2H3 cells (basophilic leukaemia cell line), were obtained from the American Type Culture Collection. Cells were cultured at 37 °C in humidified air atmosphere with 5% CO<sub>2</sub>. Cells were grown in complete media (CM) consisting of RPMI (GIBCO) supplemented with 10% heat-inactivated foetal bovine serum (FBS; GIBCO), 100 U/mL penicillin and 100 ug/mL streptomycin (GIBCO).

20

#### *Treatment of cells:*

All cell-based experiments employing inhibitors of DPP-I were performed by addition of the compound (kept below -20 °C as a 10 mM stock solution in DMSO (Sigma)) for indicated time and concentrations to sub-confluent cell cultures growing on culture dishes. All experiments were set up in triplicates. Following the treatment, cells were washed twice in ice-cold PBS (Sigma) and scraped from the culture dish. After another wash in PBS cells were lysed in ice-cold H<sub>2</sub>O containing 0.3% Triton x 100. An additional volume of buffer containing 40 mM MOPS, 2M NaCl, 2mM EDTA and 20% of glycerol was added. The cell lysate was clarified by centrifugation at 3000 *rpm* for 10 min. The resulting supernatant was transferred to eppendorf tubes. The Coomassie Plus Protein Assay (Pierce) was used to determine protein levels of the samples.

30

#### *In vitro measurement of DPP-I activity:*

35

The activity of DPP-I in cell lysates was performed in 100 mM sodium phosphate, 150 mM KCl, 1.5 mM EDTA (pH = 6) using 50 uM of H-G(Y(3NO<sub>2</sub>))GPP(K(Abz))G-OH

(Schafer-N, Denmark) as a substrate. For each measurement, cell lysate corresponding to approximately 50 µg of protein was used. All samples were prepared in duplicates.

5 Assay for determination of metabolic stability in rat liver microsomes

The assay was performed at Cerep (Seattle) according to the protocol described by Kuhn et. al. (Kuhn, W., Gieschen, H. *Drug Metab. Dispos.*, 26, 1120-1127), using rat liver microsomes (0.3 mg/mL), test compound (1 µM), NADP (1 mM), and G6P (5 mM), and G6PDHase (1 U/mL) with 0.6 % methanol/0.6 % acetonitrile in water. Incubation was performed at 37 °C in phosphate buffer (50 mM, pH = 7.4), and the samples were analyzed for remaining product ion (selected reaction monitoring) by HPLC-MS/MS (Thermo Finnigan) at 0, 15, 30, 45 and 60 min. intervals.

Results:

15

Assay	Enzyme/ species	Example 11
Primary enzymatic screening (IC <sub>50</sub> )	DPP-I	40 ± 6 nM
Secondary enzymatic screening (10 µM cutoff or IC <sub>50</sub> )	Cathepsin B	4500 nM
	Cathepsin G	> 10 µM
	Cathepsin H	3200 nM
	Cathepsin L	> 10 µM
	DPP-IV	> 10 µM
	Neutrophil elastase	> 10 µM
	Tryptase	> 10 µM
Metabolism: CYP enzymes (20 µM cutoff or IC <sub>50</sub> )	CYP1A2	> 20 µM
	CYP2C9	> 20 µM
	CYP2C19	> 20 µM
	CYP2D6	> 20 µM
	CYP3A4	600 nM
Cellular (% inhibition)	DPP-I (RBL- 2H3 cells)	54%
Metabolic stability (t <sub>1/2</sub> in min.)	Rat liver microsomes	23

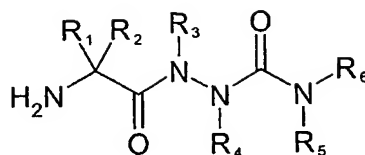
The results show that the compound tested is a selective inhibitor of DPP-I.

5 The above specification and Examples fully disclose how to make and use the compounds of the present invention. However, the present invention is not limited to the particular embodiments described hereinabove, but includes all modifications thereof within the scope of the following claims. The various references to journals, patents and other publications which are cited herein comprise the state of the art and are incorporated herein by reference as though fully set forth.

10

## CLAIMS

1. A compound of formula (I)



(I)

or a pharmaceutically acceptable salt or ester thereof,  
wherein

- 10  $R_1$  and  $R_2$  independently of each other are hydrogen,  $C_{1-6}$ alkyl,  $C_{2-6}$ alkenyl,  $C_{2-6}$ alkynyl, an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group, an unsubstituted or substituted  $C_{5-10}$ cycloalkenyl group, an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group, an unsubstituted or substituted  $C_{1-6}$ alkylaryl group but not benzyl when  $R_6$  are phenyl, an unsubstituted or substituted  $C_{1-6}$ alkylheteroaryl group, an unsubstituted or substituted aryl group, an unsubstituted or substituted heteroaryl group, an unsubstituted or substituted aryl- $C_{1-5}$ alkyl group, an unsubstituted or substituted heteroaryl- $C_{1-5}$ alkyl group, an unsubstituted or substituted  $C_{3-7}$ cycloalkyl- $C_{1-5}$ alkyl group, an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl- $C_{1-5}$ alkyl group, or  $R_1$  and  $R_2$  together form an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group or an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group;
- 20  $R_3$  is hydrogen,  $C_{1-6}$ alkyl, or  $R_3$  and  $R_1$  together form an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group or an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group, or  $R_3$  and  $R_2$  together form an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group or an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group;
- $R_4$  and  $R_5$  independently of each other are hydrogen or  $C_{1-6}$ alkyl;
- 25  $R_6$  is  $C_{1-6}$ alkyl,  $-\text{CH}(R_7)\text{CONH}(R_8)$ ,  $C_{2-6}$ alkenyl,  $C_{2-6}$ alkynyl, an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group, an unsubstituted or substituted  $C_{5-10}$ cycloalkenyl group, an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group, an unsubstituted or substituted  $C_{3-7}$ cycloalkyl- $C_{1-5}$ alkyl group, an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl- $C_{1-5}$ alkyl group, an unsubstituted or substituted  $C_{1-6}$ alkylaryl group, an unsubstituted or substituted  $C_{1-6}$ alkyl-heteroaryl group, an unsubstituted or substituted aryl group with the proviso that aryl is not substituted with a nitro group, an unsubstituted or substituted heteroaryl group, an unsubstituted or substituted aryl- $C_{1-5}$ alkyl group, an unsubstituted or substituted heteroaryl- $C_{1-5}$ alkyl group;
- 30

R<sub>7</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group, an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group or a side chain of a natural or non-natural amino acid residue;

- 5 R<sub>8</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group, an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group, a side chain of a natural or non-natural amino acid residue or a group -CH(R<sub>7</sub>)CONH(R<sub>9</sub>);

- 10 R<sub>9</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group. wherein a substituted group is substituted with one, two or three substituents independently selected from the group consisting of C<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkylthio, C<sub>1-6</sub>alkylcarbonyl, C<sub>1-6</sub>-N-alkylamide, C<sub>1-6</sub>alkoxy, dialkylamino-C<sub>1-6</sub>alkyl, amide, hydroxy, carboxy, amino, halogen, trifluoromethyl, trifluoromethoxy, trifluoromethylthio and cyano.
- 15

2. A compound according to claim 1, wherein R<sub>1</sub> and R<sub>2</sub>, independently of each other, are hydrogen, C<sub>1-6</sub>alkyl, an unsubstituted or substituted phenyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group.
- 20

3. A compound according to claim 1 or 2, wherein R<sub>1</sub> and R<sub>2</sub>, independently of each other, are selected from methyl, ethyl, propyl, butyl, iso-butyl, benzyl, 1-naphthylmethyl, 2-naphthylmethyl and (4-pyridyl)methyl.
- 25

4. A compound according to claim 1 or 2, wherein R<sub>1</sub> is C<sub>1-6</sub>alkyl and R<sub>2</sub> is H.

5. A compound according to claim 1, wherein R<sub>1</sub> and R<sub>2</sub> together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub> heterocycloalkyl group.
- 30

6. A compound according to claim 5, wherein R<sub>1</sub> and R<sub>2</sub> together form an unsubstituted or substituted cyclohexyl group.

- 35 7. A compound according to any of the preceding claims, wherein R<sub>3</sub> is hydrogen or methyl.

8. A compound according to any of the claims 1-6, wherein  $R_1$  and  $R_3$  together form an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group or an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group.
- 5 9. A compound according to claim 1, wherein  $R_2$  and  $R_3$  together form an unsubstituted or substituted  $C_{3-10}$ cycloalkyl group or an unsubstituted or substituted  $C_{3-7}$ heterocycloalkyl group.
- 10 10. A compound according to any of the preceding claims, wherein  $R_4$  is hydrogen or methyl.
11. A compound according to any of the preceding claims, wherein  $R_5$  is hydrogen or methyl.
- 15 12. A compound according to any of claims 1-7, 10-11, wherein at least one of  $R_3$  and  $R_4$  and  $R_5$  is hydrogen.
13. A compound according to claim 12, wherein  $R_3$  and  $R_4$  and  $R_5$  is hydrogen.
- 20 14. A compound according to any of the preceding claims, wherein  $R_6$  is -  $CH(R_7)CONH(R_8)$ ,  $C_{1-6}$ alkyl, an unsubstituted or substituted  $C_{1-6}$ alkylaryl group or an unsubstituted or substituted  $C_{1-6}$ alkylheteroaryl group.
- 25 15. A compound according to any of the preceding claims, wherein  $R_6$  is an unsubstituted or substituted benzyl group.
16. A compound according to any of claims 1-14, wherein  $R_6$  is the group -  $CH(R_7)CONH(R_8)$ .
- 30 17. A compound according to claim 1, wherein  $R_6$  is selected from 2S-3-phenylpropan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-cyclohexylpropan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-butan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-2-
- 35



phenylethan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide, 2S-N-[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-3-(p-chlorophenyl)propan-2-yl-amide, 2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide, 5 2S-N-[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide, 2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(p-hydroxyphenyl)propan-2-yl-amide, 2S-N-[2S-4-methylpentan-2-yl-amide]-3, (benzo[b]thiophen-3-yl)propan-2-yl-amide, 2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(p-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide, 2-phenylethyl, 2-(p-hydroxyphenyl)ethyl, benzyl, o-chlorobenzyl, 2-furfuryl, 2-(3-indolyl)ethyl, 1- 15 naphthylmethyl, 4-piperonyl, p-chlorobenzyl, 2-(thiophen-2-yl)ethyl, m-fluorobenzyl, o-fluorobenzyl, p-fluorobenzyl, o-trifluoromethylbenzyl, m-chlorobenzyl, o-methoxybenzyl, o-methylbenzyl, alpha-methylbenzyl, o-(o-hydroxymethylphenylthio)benzyl, o-bromobenzyl, 2-chloro-6-fluorobenzyl, o-ethoxybenzyl, [1,1'-biphenyl-4-yl]methyl, [1,1'-biphenyl-2-yl]methyl, p-[thiophen-2-yl]benzyl, o-[N-morpholinyl]benzyl, o-[N- 20 piperidinyl]benzyl and o-methylthiobenzyl.

18. A compound according to any of claims 1-14, wherein R<sub>6</sub> is an unsubstituted or substituted [1,1'-biphenyl-2-yl]methyl group or an unsubstituted or substituted [1,1'-biphenyl-4-yl]methyl group.

19. A compound according to any of claims 1-14, 16, wherein R<sub>7</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group, an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group.

20. A compound according to claim 19, wherein R<sub>7</sub> is an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group.

21. A compound according to claim 20, wherein R<sub>7</sub> is 2-phenylethyl.

22. A compound according to any of claims 1-14, 16-21, wherein  $R_8$  is hydrogen,  $-\text{CH}(R_7)\text{CONH}(R_9)$  or an unsubstituted or substituted  $\text{C}_{1-6}$ alkyl group.
23. A compound according to claim 22, wherein  $R_8$  is  $-\text{CH}(R_7)\text{CONH}(R_9)$  and  $R_7$  is an  
5 unsubstituted or substituted  $\text{C}_{1-6}$ alkylaryl group or an unsubstituted or substituted  $\text{C}_{1-6}$ alkyl-heteroaryl group.
24. A compound of claim 23, wherein  $R_7$  is (indol-3-yl)methyl or (*m*-  
10 fluorophenyl)methyl.
25. A compound according to claim 23, wherein  $R_9$  is hydrogen or an unsubstituted or substituted  $\text{C}_{1-6}$ alkyl group.
26. A compound according to claim 25, wherein  $R_9$  is hydrogen.
27. A compound according to claim 1 selected from the group consisting of  
15 1-(2*S*-2-Aminopropionyl)-4-(2*S*-3-phenylpropan-2-yl-amide)semicarbazide;  
1-(aminoacetyl)-4-(2*S*-3-phenylpropan-2-yl-amide)semicarbazide;  
1-(2*S*-2-aminopropionyl)-1-methyl-4-(2*S*-3-phenylpropan-2-yl-amide)semicarbazide;  
20 1-(1-aminocyclohexane-1-carbonyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-  
3-cyclohexylpropan-2-yl-amide]semicarbazide;  
1-(1-aminocyclohexane-1-carbonyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-  
4-phenylbutan-2-yl-amide]semicarbazide;  
1-(2*S*-2-aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-  
25 2-yl-amide]semicarbazide;  
1-(2*S*-2-aminopropanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-3-  
phenylpropan-2-yl-amide]semicarbazide;  
1-(2*S*-3-naphthyl-2-propanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-  
methylbutan-2-yl-amide]semicarbazide;  
30 1-[2*S*-2-amino-3-(4-pyridyl)propanoyl]-4-[2*S*-*N*-(2*S*-3-(indol-3-yl)propan-2-yl-amide)-  
butan-2-yl-amide]semicarbazide;  
1-[2*S*-2-amino-3-(4-pyridyl)propanoyl]-4-[2*S*-*N*-(2*S*-3-(indol-3-yl)propan-2-yl-amide)-2-  
phenylethan-2-yl-amide]semicarbazide;  
1-(2*S*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-  
35 yl-amide]semicarbazide;

- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;
- 5 1-(2S-2-Aminopentanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-amino-4-phenylbutanoyl)-4-[2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;
- 1-(2S-2-aminopentanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;
- 10 1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(*p*-hydroxyphenyl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-4-methylpentan-2-yl-amide]-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]semicarbazide;
- 15 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 20 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*p*-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminopentanoyl)-4-[2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 25 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-aminopropionyl)-4-(2-phenylethyl)semicarbazide;
- 30 1-(2S-2-aminopropionyl)-4-(2-phenylethyl)-4-methylsemicarbazide;
- 1-(2S-2-aminopropionyl)-4-[2-(*p*-hydroxyphenyl)ethyl]semicarbazide;
- 1-(2S-2-aminopropionyl)-4-benzylsemicarbazide;
- 1-(2S-2-aminopropionyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2S-2-aminopropionyl)-4-(2-furfuryl)semicarbazide;
- 35 1-(2S-2-aminopropionyl)-4-[2-(3-indolyl)ethyl]semicarbazide;
- 1-(2S-2-aminopropionyl)-4-(1-naphthylmethyl)semicarbazide;

- 1-(2S-2-aminopropionyl)-4-piperonylsemicarbazide;  
 1-(2S-2-aminopropionyl)-4-(*p*-chlorobenzyl)semicarbazide;  
 1-(2S-2-aminopropionyl)-4-[2-(thiophen-2-yl)ethyl]semicarbazide;  
 1-(2S-2-aminopropionyl)-4-(*m*-fluorobenzyl)semicarbazide;  
 5 1-(2S-2-aminopropionyl)-4-(*o*-fluorobenzyl)semicarbazide;  
 1-(2S-2-aminopropionyl)-4-(*p*-fluorobenzyl)semicarbazide;  
 1-(2S-2-aminopropionyl)-4-(*o*-trifluoromethylbenzyl)semicarbazide;  
 1-(2S-2-aminopropionyl)-4-(*m*-chlorobenzyl)semicarbazide;  
 1-(2S-2-aminopropionyl)-4-(*o*-methoxybenzyl)semicarbazide;  
 10 1-(2S-2-aminopropionyl)-4-(*o*-methylbenzyl)semicarbazide;  
 1-(2S-2-aminopropionyl)-4-( $\alpha$ -methylbenzyl)semicarbazide;  
 1-(2S-2-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
 1-(2S-2-aminovaleryl)-4-(*o*-chlorobenzyl)semicarbazide;  
 1-(2S-2-aminohexanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
 15 1-(2S-2-aminoisocaproyl)-4-(*o*-chlorobenzyl)semicarbazide;  
 1-(1-aminocyclohexane-1-carbonyl)-4-(*o*-chlorobenzyl)semicarbazide;  
 1-(2S-2-amino-3-phenyl-propionyl)-4-(*o*-chlorobenzyl)semicarbazide;  
 1,1-(2S-1,4-cyclo-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
 1,1-(2R-1,4-cyclo-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
 20 1-(2S-2-Aminobutanoyl)-4-[2S-*N*-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-[*o*-(*o*-hydroxymethylphenylthio)benzyl]semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-(*o*-bromobenzyl)semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-(2-chloro-6-fluorobenzyl)semicarbazide;  
 25 1-(2S-2-Aminobutanoyl)-4-(*o*-ethoxybenzyl)semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-([1,1'-biphenyl-4-yl]methyl)semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-([1,1'-biphenyl-2-yl]methyl)semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-(*p*-[thiophen-2-yl]benzyl)semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-(*o*-[*N*-morpholinyl]benzyl)semicarbazide;  
 30 1-(2S-2-Aminobutanoyl)-4-(1-naphthylmethyl)semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-(*o*-[*N*-piperidinyl]benzyl)semicarbazide; and  
 1-(2S-2-Aminobutanoyl)-4-(*o*-methylthiobenzyl)semicarbazide.
28. A compound according to claim 1 selected from the group consisting of
- 35 1-(2R-2-aminopropionyl)-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
 1-(2R-2-aminopropionyl)-1-methyl-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;

- 1-(2*R*-2-aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-aminopropanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;
- 5 1-(2*R*-3-naphthyl-2-propanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide]semicarbazide;
- 1-[2*R*-2-amino-3-(4-pyridyl)propanoyl]-4-[2*S*-*N*-(2*S*-3-(indol-3-yl)propan-2-yl-amide)-butan-2-yl-amide]semicarbazide;
- 1-[2*R*-2-amino-3-(4-pyridyl)propanoyl]-4-[2*S*-*N*-(2*S*-3-(indol-3-yl)propan-2-yl-amide)-2-phenylethan-2-yl-amide]semicarbazide;
- 10 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide]semicarbazide;
- 15 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminopentanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-amino-4-phenylbutanoyl)-4-[2*S*-*N*-[2*S*-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;
- 20 1-(2*R*-2-aminopentanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(*p*-hydroxyphenyl)propan-2-yl-amide]semicarbazide;
- 25 1-(2*R*-2-aminobutanoyl)-4-[2*S*-*N*-[2*S*-4-methylpentan-2-yl-amide]-3-(benzo[*b*]thiophen-3-yl)propan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 30 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(benzo[*b*]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*p*-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 35 1-(2*R*-2-Aminopentanoyl)-4-[2*S*-*N*-[2*S*-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;

- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide]semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;
- 5 1-(2*R*-2-aminopropionyl)-4-(2-phenylethyl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(2-phenylethyl)-4-methylsemicarbazide;  
1-(2*R*-2-aminopropionyl)-4-[2-(*p*-hydroxyphenyl)ethyl]semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-benzylsemicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 10 1-(2*R*-2-aminopropionyl)-4-(2-furfuryl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-[2-(3-indolyl)ethyl]semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(1-naphthylmethyl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-piperonylsemicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(*p*-chlorobenzyl)semicarbazide;
- 15 1-(2*R*-2-aminopropionyl)-4-[2-(thiophen-2-yl)ethyl]semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(*m*-fluorobenzyl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(*o*-fluorobenzyl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(*p*-fluorobenzyl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(*o*-trifluoromethylbenzyl)semicarbazide;
- 20 1-(2*R*-2-aminopropionyl)-4-(*m*-chlorobenzyl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(*o*-methoxybenzyl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(*o*-methylbenzyl)semicarbazide;  
1-(2*R*-2-aminopropionyl)-4-(alpha-methylbenzyl)semicarbazide;  
1-(2*R*-2-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 25 1-(2*R*-2-aminovaleryl)- 4-(*o*-chlorobenzyl)semicarbazide;  
1-(2*R*-2-aminohexanoyl)- 4-(*o*-chlorobenzyl)semicarbazide;  
1-(2*R*-2-aminoisocaproyl)- 4-(*o*-chlorobenzyl)semicarbazide;  
1-(2*R*-2-amino-3-phenyl-propionyl)- 4-(*o*-chlorobenzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 30 1-(2*R*-2-Aminobutanoyl)-4-[*o*-(*o*-hydroxymethylphenylthio)benzyl]semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-bromobenzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(2-chloro-6-fluorobenzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-ethoxybenzyl)semicarbazide;
- 35 1-(2*R*-2-Aminobutanoyl)-4-([1,1'-biphenyl-4-yl]methyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-([1,1'-biphenyl-2-yl]methyl)semicarbazide;

- 1-(2*R*-2-Aminobutanoyl)-4-(*p*-[thiophen-2-yl]benzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-[*N*-morpholinyl]benzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(1-naphthylmethyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-[*N*-piperidinyl]benzyl)semicarbazide; and  
5 1-(2*R*-2-Aminobutanoyl)-4-(*o*-methylthiobenzyl)semicarbazide.

29. A compound according to any of the preceding claims, which in the DPP-I assay exhibits an IC<sub>50</sub> value of less than 500 μM such as, e.g., less than 100 μM, less than 50 μM, less than 1 μM, less than 500 nM, less than 100 nM or less than 50 nM.

10

30. A compound according to any of the preceding claims for use in medicine.

31. A compound according to claim 30 for use as a protease inhibitor.

- 15 32. A compound according to claim 31 for use as a cysteine protease inhibitor.

33. A compound according to any of claims 30-32 for use in the treatment, prophylaxis and/or diagnosis of inflammation, type2 diabetes, asthma, severe influenza, respiratory syncytial virus infection, CD8 T cell inhibition, inflammatory bowel diseases, psoriasis,  
20 atopic dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease, periodontitis, rheumatoid arthritis, Huntington's disease, Chagas' disease, Alzheimer's disease, sepsis or for application in target cell apoptosis.

- 25 34. A pharmaceutical composition comprising, as an active substance, a compound as defined in any of claims 1-33 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

35. A pharmaceutical composition according to claim 34 in unit dosage form, comprising from about 1 μg to about 1000 mg such as, e.g., from about 10 μg to about  
30 500 mg, from about 0.05 to about 100 mg or from about 0.1 to about 50 mg, of the active substance.

36. A pharmaceutical composition according to claim 34 or 35 for oral, nasal, transdermal, pulmonal or parenteral administration.

35

37. A method for the treatment of ailments, the method comprising administering to a subject in need thereof an effective amount of a compound as defined in any of claims 1-33 or of a composition as defined in any of claims 34-36.

5 38. The method according to claim 37, wherein the effective amount of the compound is in a range of from about 1  $\mu$ g to about 1000 mg such as, e.g., from about 10  $\mu$ g to about 500 mg, from about 0.05 to about 100 mg or from about 0.1 to about 50 mg per day.

10 39. Use of a compound as defined in any of claims 1-33 for the preparation of a medicament.

40. Use of a compound as defined in any of claims 1-33 for the preparation of a medicament for treatment, prophylaxis and/or diagnosis of inflammation, type2  
15 diabetes, asthma, severe influenza, respiratory syncytial virus infection, CD8 T cell inhibition, inflammatory bowel diseases, psoriasis, atopic dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease, periodontitis, rheumatoid arthritis, Huntington's disease, Chagas' disease, Alzheimer's disease, sepsis or for application in target cell apoptosis.

20

41. A method for modulating DPP-I levels in a subject in need thereof comprising administering to said subject an amount of a compound as defined in any of claims 1-33 or a composition as defined in any of claims 34-36 in an amount effective to modulate said DPP-I levels in said subject.

25

42. The method according to claim 41, wherein said DPPI is inhibited.

43. The method according to claim 42, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Cathepsin B})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or  
30 more, 75 or more or 100 or more.

44. The method according to claim 42 or 43, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Cathepsin G})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.

35

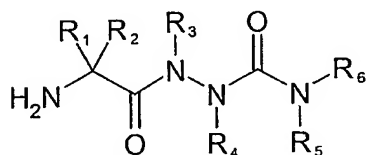


45. The method according to any of claim 42-44, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Cathepsin H})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
- 5    46. The method according to any of claim 42-45, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Cathepsin L})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
- 10    47. The method according to any of claim 42-46, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{DPP-IV})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
- 15    48. The method according to any of claim 42-47, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Neutrophil elastase})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
49. The method according to any of claim 42-48, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Tryptase})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.

## AMENDED CLAIMS

[Received by the International Bureau on 06 October 2004 (06.10.2004) ;  
original claim 1, amended ; remaining claims, unchanged]

1. A compound of formula (I)



(I)

or a pharmaceutically acceptable salt or ester thereof,

wherein

- 10 R<sub>1</sub> and R<sub>2</sub> independently of each other are hydrogen, C<sub>1-6</sub>alkyl, C<sub>2-6</sub>alkenyl, C<sub>2-6</sub>alkynyl, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>5-10</sub>cycloalkenyl group, an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group but not benzyl when R<sub>6</sub> are phenyl, an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group, an unsubstituted or substituted aryl group, an unsubstituted or substituted heteroaryl group, an unsubstituted or substituted aryl-C<sub>1-5</sub>alkyl group, an unsubstituted or substituted heteroaryl-C<sub>1-5</sub>alkyl group, an unsubstituted or substituted C<sub>3-7</sub>cycloalkyl-C<sub>1-5</sub>alkyl group, an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl-C<sub>1-5</sub>alkyl group, or R<sub>1</sub> and R<sub>2</sub> together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl group;
- 20 R<sub>3</sub> is hydrogen, C<sub>1-6</sub>alkyl, or R<sub>3</sub> and R<sub>1</sub> together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl group, or R<sub>3</sub> and R<sub>2</sub> together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl group;
- R<sub>4</sub> and R<sub>5</sub> independently of each other are hydrogen or C<sub>1-6</sub>alkyl;
- 25 R<sub>6</sub> is C<sub>1-6</sub>alkyl, -CH(R<sub>7</sub>)CONH(R<sub>8</sub>), C<sub>2-6</sub>alkenyl, C<sub>2-6</sub>alkynyl, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>5-10</sub>cycloalkenyl group, an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl group, an unsubstituted or substituted C<sub>3-7</sub>cycloalkyl-C<sub>1-5</sub>alkyl group, an unsubstituted or substituted C<sub>3-7</sub>heterocycloalkyl-C<sub>1-5</sub>alkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group, an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group, an unsubstituted or substituted aryl group, an unsubstituted or substituted heteroaryl group, an unsubstituted or substituted aryl-C<sub>1-5</sub>alkyl group, an unsubstituted or substituted heteroaryl-C<sub>1-5</sub>alkyl group;
- 30 group;

R<sub>7</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group, an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group or a side chain of a natural or non-natural amino acid residue;

- 5 R<sub>8</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group, an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group, a side chain of a natural or non-natural amino acid residue or a group -CH(R<sub>7</sub>)CONH(R<sub>9</sub>);

- 10 R<sub>9</sub> is hydrogen, an unsubstituted or substituted C<sub>1-6</sub>alkyl group, an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group, wherein a substituted group is substituted with one, two or three substituents independently selected from the group consisting of C<sub>1-6</sub>alkyl, C<sub>1-6</sub>alkylthio, C<sub>1-6</sub>alkylcarbonyl, C<sub>1-6</sub>-N-alkylamide, C<sub>1-6</sub>alkoxy, dialkylamino-C<sub>1-6</sub>alkyl, amide, hydroxy, carboxy, amino, halogen, trifluoromethyl, trifluoromethoxy, trifluoromethylthio and cyano;

- 15 with the proviso that when R<sub>1</sub> is hydrogen, R<sub>2</sub> is 4-hydroxybenzyl, R<sub>3</sub> is hydrogen, R<sub>4</sub> is methyl, R<sub>5</sub> is hydrogen, R<sub>6</sub> is -CH(R<sub>7</sub>)CONH(R<sub>8</sub>), wherein R<sub>7</sub> is hydrogen, R<sub>8</sub> is -CH(R<sub>7</sub>)CONH(R<sub>9</sub>), wherein R<sub>7</sub> is benzyl and R<sub>9</sub> is 1-substituted isopentyl, then a substituent is not carboxy;
- 20 with the proviso that when R<sub>1</sub> is hydrogen, R<sub>2</sub> is sec-butyl, R<sub>3</sub> is hydrogen, R<sub>4</sub> is hydrogen, R<sub>5</sub> is hydrogen, R<sub>6</sub> is -CH(R<sub>7</sub>)CONH(R<sub>8</sub>), wherein R<sub>7</sub> is isobutyl and R<sub>8</sub> is -CH(R<sub>7</sub>)CONH(R<sub>9</sub>), wherein R<sub>7</sub> is a 2-mercaptomethyl substituted ethyl, then R<sub>9</sub> is not hydrogen.

- 25 2. A compound according to claim 1, wherein R<sub>1</sub> and R<sub>2</sub>, independently of each other, are hydrogen, C<sub>1-6</sub>alkyl, an unsubstituted or substituted phenyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group.

- 30 3. A compound according to claim 1 or 2, wherein R<sub>1</sub> and R<sub>2</sub>, independently of each other, are selected from methyl, ethyl, propyl, butyl, iso-butyl, benzyl, 1-naphthylmethyl, 2-naphthylmethyl and (4-pyridyl)methyl.

4. A compound according to claim 1 or 2, wherein R<sub>1</sub> is C<sub>1-6</sub>alkyl and R<sub>2</sub> is H.

5. A compound according to claim 1, wherein R<sub>1</sub> and R<sub>2</sub> together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub> heterocycloalkyl group.
- 5 6. A compound according to claim 5, wherein R<sub>1</sub> and R<sub>2</sub> together form an unsubstituted or substituted cyclohexyl group.
7. A compound according to any of the preceding claims, wherein R<sub>3</sub> is hydrogen or methyl.
- 10 8. A compound according to any of the claims 1-6, wherein R<sub>1</sub> and R<sub>3</sub> together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub> heterocycloalkyl group.
- 15 9. A compound according to claim 1, wherein R<sub>2</sub> and R<sub>3</sub> together form an unsubstituted or substituted C<sub>3-10</sub>cycloalkyl group or an unsubstituted or substituted C<sub>3-7</sub> heterocycloalkyl group.
10. A compound according to any of the preceding claims, wherein R<sub>4</sub> is hydrogen or methyl.
- 20 11. A compound according to any of the preceding claims, wherein R<sub>5</sub> is hydrogen or methyl.
12. A compound according to any of claims 1-7, 10-11, wherein at least one of R<sub>3</sub> and R<sub>4</sub> and R<sub>5</sub> is hydrogen.
13. A compound according to claim 12, wherein R<sub>3</sub> and R<sub>4</sub> and R<sub>5</sub> is hydrogen.
14. A compound according to any of the preceding claims, wherein R<sub>6</sub> is - CH(R<sub>7</sub>)CONH(R<sub>8</sub>), C<sub>1-6</sub>alkyl, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group.
15. A compound according to any of the preceding claims, wherein R<sub>6</sub> is an unsubstituted or substituted benzyl group.
- 35

16. A compound according to any of claims 1-14, wherein  $R_6$  is the group -  
CH( $R_7$ )CONH( $R_8$ ).

17. A compound according to claim 1, wherein  $R_6$  is selected from 2S-3-phenylpropan-  
5 2-yl-amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-cyclohexylpropan-2-yl-  
amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-  
N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide, 2S-N-[2S-3-(m-  
fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide, 2S-N-[2S-3-(indol-3-  
yl)propan-2-yl-amide]-butan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-2-  
10 phenylethan-2-yl-amide, 2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-  
amide, 2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide,  
2S-N-[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-3-(p-chlorophenyl)propan-2-yl-  
amide, 2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide,  
2S-N-[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-  
15 amide, 2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(p-hydroxyphenyl)propan-  
2-yl-amide, 2S-N-[2S-4-methylpentan-2-yl-amide]-3, (benzo[b]thiophen-3-yl)propan-2-  
yl-amide, 2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-  
[2S-3-(m-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-  
(benzo[b]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(p-  
20 chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide, 2S-N-[2S-3-(m-  
fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide, 2S-N-[2S-3-(m-  
fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide, 2-phenylethyl,  
2-(p-hydroxyphenyl)ethyl, benzyl, o-chlorobenzyl, 2-furfuryl, 2-(3-indolyl)ethyl, 1-  
naphthylmethyl, 4-piperonyl, p-chlorobenzyl, 2-(thiophen-2-yl)ethyl, m-fluorobenzyl, o-  
25 fluorobenzyl, p-fluorobenzyl, o-trifluoromethylbenzyl, m-chlorobenzyl, o-methoxybenzyl,  
o-methylbenzyl, alpha-methylbenzyl, o-(o-hydroxymethylphenylthio)benzyl, o-  
bromobenzyl, 2-chloro-6-fluorobenzyl, o-ethoxybenzyl, [1,1'-biphenyl-4-yl]methyl, [1,1'-  
biphenyl-2-yl]methyl, p-[thiophen-2-yl]benzyl, o-[N-morpholinyl]benzyl, o-[N-  
piperidinyl]benzyl and o-methylthiobenzyl.

30

18. A compound according to any of claims 1-14, wherein  $R_6$  is an unsubstituted or  
substituted [1,1'-biphenyl-2-yl]methyl group or an unsubstituted or substituted [1,1'-  
biphenyl-4-yl]methyl group.

35

19. A compound according to any of claims 1-14, 16, wherein  $R_7$  is hydrogen, an  
unsubstituted or substituted  $C_{1-6}$ alkyl group, an unsubstituted or substituted  $C_3$ .

cycloalkyl group, an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group, an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group.

20. A compound according to claim 19, wherein R<sub>7</sub> is an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkylheteroaryl group.

21. A compound according to claim 20, wherein R<sub>7</sub> is 2-phenylethyl.

22. A compound according to any of claims 1-14, 16-21, wherein R<sub>8</sub> is hydrogen, –CH(R<sub>7</sub>)CONH(R<sub>9</sub>) or an unsubstituted or substituted C<sub>1-6</sub>alkyl group.

23. A compound according to claim 22, wherein R<sub>8</sub> is –CH(R<sub>7</sub>)CONH(R<sub>9</sub>) and R<sub>7</sub> is an unsubstituted or substituted C<sub>1-6</sub>alkylaryl group or an unsubstituted or substituted C<sub>1-6</sub>alkyl-heteroaryl group.

24. A compound of claim 23, wherein R<sub>7</sub> is (indol-3-yl)methyl or (m-fluorophenyl)methyl.

25. A compound according to claim 23, wherein R<sub>9</sub> is hydrogen or an unsubstituted or substituted C<sub>1-6</sub>alkyl group.

26. A compound according to claim 25, wherein R<sub>9</sub> is hydrogen.

27. A compound according to claim 1 selected from the group consisting of  
 1-(2S-2-Aminopropionyl)-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
 1-(aminoacetyl)-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
 1-(2S-2-aminopropionyl)-1-methyl-4-(2S-3-phenylpropan-2-yl-amide)semicarbazide;  
 1-(1-aminocyclohexane-1-carbonyl)-4-[2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-3-cyclohexylpropan-2-yl-amide]semicarbazide;  
 1-(1-aminocyclohexane-1-carbonyl)-4-[2S-N-[2S-3-(m-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;  
 1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;  
 1-(2S-2-aminopropanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;

- 1-(2S-3-naphthyl-2-propanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide]semicarbazide;
- 1-[2S-2-amino-3-(4-pyridyl)propanoyl]-4-[2S-N-(2S-3-(indol-3-yl)propan-2-yl-amide)-butan-2-yl-amide]semicarbazide;
- 5 1-[2S-2-amino-3-(4-pyridyl)propanoyl]-4-[2S-N-(2S-3-(indol-3-yl)propan-2-yl-amide)-2-phenylethan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide]semicarbazide;
- 10 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminopentanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;
- 15 1-(2S-2-amino-4-phenylbutanoyl)-4-[2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;
- 1-(2S-2-aminopentanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(*p*-hydroxyphenyl)propan-2-yl-amide]semicarbazide;
- 20 1-(2S-2-aminobutanoyl)-4-[2S-N-[2S-4-methylpentan-2-yl-amide]-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 25 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(benzo[b]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*p*-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 30 1-(2S-2-Aminopentanoyl)-4-[2S-N-[2S-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide]semicarbazide;
- 35 1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;

- 1-(2S-2-aminopropionyl)-4-(2-phenylethyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(2-phenylethyl)-4-methylsemicarbazide;  
1-(2S-2-aminopropionyl)-4-[2-(*p*-hydroxyphenyl)ethyl]semicarbazide;  
1-(2S-2-aminopropionyl)-4-benzylsemicarbazide;  
5 1-(2S-2-aminopropionyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(2-furfuryl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-[2-(3-indolyl)ethyl]semicarbazide;  
1-(2S-2-aminopropionyl)-4-(1-naphthylmethyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-piperonylsemicarbazide;  
10 1-(2S-2-aminopropionyl)-4-(*p*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-[2-(thiophen-2-yl)ethyl]semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*m*-fluorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*o*-fluorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*p*-fluorobenzyl)semicarbazide;  
15 1-(2S-2-aminopropionyl)-4-(*o*-trifluoromethylbenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*m*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*o*-methoxybenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(*o*-methylbenzyl)semicarbazide;  
1-(2S-2-aminopropionyl)-4-(alpha-methylbenzyl)semicarbazide;  
20 1-(2S-2-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminovaleryl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminohexanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-aminoisocaproyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(1-aminocyclohexane-1-carbonyl)-4-(*o*-chlorobenzyl)semicarbazide;  
25 1-(2S-2-amino-3-phenyl-propionyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1,1-(2S-1,4-cyclo-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1,1-(2R-1,4-cyclo-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-[2S-N-[2S-3-(*m*-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;  
30 1-(2S-2-Aminobutanoyl)-4-[*o*-(*o*-hydroxymethylphenylthio)benzyl]semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(*o*-bromobenzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(2-chloro-6-fluorobenzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(*o*-ethoxybenzyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-([1,1'-biphenyl-4-yl]methyl)semicarbazide;  
35 1-(2S-2-Aminobutanoyl)-4-([1,1'-biphenyl-2-yl]methyl)semicarbazide;  
1-(2S-2-Aminobutanoyl)-4-(*p*-[thiophen-2-yl]benzyl)semicarbazide;



1-(2S-2-Aminobutanoyl)-4-(*o*-[*N*-morpholinyl]benzyl)semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-(1-naphthylmethyl)semicarbazide;  
 1-(2S-2-Aminobutanoyl)-4-(*o*-[*N*-piperidinyl]benzyl)semicarbazide; and  
 1-(2S-2-Aminobutanoyl)-4-(*o*-methylthiobenzyl)semicarbazide.

5

28. A compound according to claim 1 selected from the group consisting of

- 1-(2*R*-2-aminopropionyl)-4-(2*S*-3-phenylpropan-2-yl-amide)semicarbazide;  
 1-(2*R*-2-aminopropionyl)-1-methyl-4-(2*S*-3-phenylpropan-2-yl-amide)semicarbazide;  
 1-(2*R*-2-aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-  
 10 2-yl-amide]semicarbazide;  
 1-(2*R*-2-aminopropanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;  
 1-(2*R*-3-naphthyl-2-propanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-methylbutan-2-yl-amide]semicarbazide;  
 15 1-[2*R*-2-amino-3-(4-pyridyl)propanoyl]-4-[2*S*-*N*-(2*S*-3-(indol-3-yl)propan-2-yl-amide)-butan-2-yl-amide]semicarbazide;  
 1-[2*R*-2-amino-3-(4-pyridyl)propanoyl]-4-[2*S*-*N*-(2*S*-3-(indol-3-yl)propan-2-yl-amide)-2-phenylethan-2-yl-amide]semicarbazide;  
 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(indol-3-yl)propan-2-yl-amide]-4-phenylbutan-2-  
 20 yl-amide]semicarbazide;  
 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(indol-3-yl)propan-2-yl-amide]semicarbazide;  
 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;  
 25 1-(2*R*-2-Aminopentanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(*p*-chlorophenyl)propan-2-yl-amide]semicarbazide;  
 1-(2*R*-2-amino-4-phenylbutanoyl)-4-[2*S*-*N*-[2*S*-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-phenylpropan-2-yl-amide]semicarbazide;  
 1-(2*R*-2-aminopentanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-methoxyphenyl)propan-2-yl-amide]-3-(1,1'-  
 30 biphenyl-4-yl)propan-2-yl-amide]semicarbazide;  
 1-(2*R*-2-aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]-3-(*p*-hydroxyphenyl)propan-2-yl-amide]semicarbazide;  
 1-(2*R*-2-aminobutanoyl)-4-[2*S*-*N*-[2*S*-4-methylpentan-2-yl-amide]-3-(benzo[*b*]thiophen-3-yl)propan-2-yl-amide]semicarbazide;  
 35 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;

- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-methoxyphenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(benzo[*b*]thiophen-3-yl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 5 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*p*-chlorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminopentanoyl)-4-[2*S*-*N*-[2*S*-3-(2-naphthyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(2-naphthyl)propan-2-yl-amide]semicarbazide;
- 10 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-3-(1,1'-biphenyl-4-yl)propan-2-yl-amide]semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(2-phenylethyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(2-phenylethyl)-4-methylsemicarbazide;
- 15 1-(2*R*-2-aminopropionyl)-4-[2-(*p*-hydroxyphenyl)ethyl]semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-benzylsemicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(2-furfuryl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-[2-(3-indolyl)ethyl]semicarbazide;
- 20 1-(2*R*-2-aminopropionyl)-4-(1-naphthylmethyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-piperonylsemicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*p*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-[2-(thiophen-2-yl)ethyl]semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*m*-fluorobenzyl)semicarbazide;
- 25 1-(2*R*-2-aminopropionyl)-4-(*o*-fluorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*p*-fluorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*o*-trifluoromethylbenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*m*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(*o*-methoxybenzyl)semicarbazide;
- 30 1-(2*R*-2-aminopropionyl)-4-(*o*-methylbenzyl)semicarbazide;
- 1-(2*R*-2-aminopropionyl)-4-(alpha-methylbenzyl)semicarbazide;
- 1-(2*R*-2-aminobutanoyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminovaleryl)-4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-aminohexanoyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 35 1-(2*R*-2-aminoisocaproyl)-4-(*o*-chlorobenzyl)semicarbazide;
- 1-(2*R*-2-amino-3-phenyl-propionyl)-4-(*o*-chlorobenzyl)semicarbazide;

- 1-(2*R*-2-Aminobutanoyl)-4-[2*S*-*N*-[2*S*-3-(*m*-fluorophenyl)propan-2-yl-amide]-4-phenylbutan-2-yl-amide]semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-[*o*-(*o*-hydroxymethylphenylthio)benzyl]semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-bromobenzyl)semicarbazide;  
5 1-(2*R*-2-Aminobutanoyl)-4-(2-chloro-6-fluorobenzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-ethoxybenzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-([1,1'-biphenyl-4-yl]methyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-([1,1'-biphenyl-2-yl]methyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*p*-[thiophen-2-yl]benzyl)semicarbazide;  
10 1-(2*R*-2-Aminobutanoyl)-4-(*o*-[*N*-morpholinyl]benzyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(1-naphthylmethyl)semicarbazide;  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-[*N*-piperidiny]benzyl)semicarbazide; and  
1-(2*R*-2-Aminobutanoyl)-4-(*o*-methylthiobenzyl)semicarbazide.
- 15 29. A compound according to any of the preceding claims, which in the DPP-I assay exhibits an IC<sub>50</sub> value of less than 500 μM such as, e.g., less than 100 μM, less than 50 μM, less than 1 μM, less than 500 nM, less than 100 nM or less than 50 nM.
30. A compound according to any of the preceding claims for use in medicine.
- 20 31. A compound according to claim 30 for use as a protease inhibitor.
32. A compound according to claim 31 for use as a cysteine protease inhibitor.
- 25 33. A compound according to any of claims 30-32 for use in the treatment, prophylaxis and/or diagnosis of inflammation, type2 diabetes, asthma, severe influenza, respiratory syncytial virus infection, CD8 T cell inhibition, inflammatory bowel diseases, psoriasis, atopic dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease, periodontitis, rheumatoid arthritis, Huntington's disease, Chagas' disease, Alzheimer's  
30 disease, sepsis or for application in target cell apoptosis.
34. A pharmaceutical composition comprising, as an active substance, a compound as defined in any of claims 1-33 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

35. A pharmaceutical composition according to claim 34 in unit dosage form, comprising from about 1 µg to about 1000 mg such as, e.g., from about 10 µg to about 500 mg, from about 0.05 to about 100 mg or from about 0.1 to about 50 mg, of the active substance.

5

36. A pharmaceutical composition according to claim 34 or 35 for oral, nasal, transdermal, pulmonal or parenteral administration.

10

37. A method for the treatment of ailments, the method comprising administering to a subject in need thereof an effective amount of a compound as defined in any of claims 1-33 or of a composition as defined in any of claims 34-36.

15

38. The method according to claim 37, wherein the effective amount of the compound is in a range of from about 1 µg to about 1000 mg such as, e.g., from about 10 µg to about 500 mg, from about 0.05 to about 100 mg or from about 0.1 to about 50 mg per day.

20

39. Use of a compound as defined in any of claims 1-33 for the preparation of a medicament.

25

40. Use of a compound as defined in any of claims 1-33 for the preparation of a medicament for treatment, prophylaxis and/or diagnosis of inflammation, type2 diabetes, asthma, severe influenza, respiratory syncytial virus infection, CD8 T cell inhibition, inflammatory bowel diseases, psoriasis, atopic dermatitis, Papillon Lefevre syndrome, Haim Munk syndrome, gum disease, periodontitis, rheumatoid arthritis, Huntington's disease, Chagas' disease, Alzheimer's disease, sepsis or for application in target cell apoptosis.

30

41. A method for modulating DPP-I levels in a subject in need thereof comprising administering to said subject an amount of a compound as defined in any of claims 1-33 or a composition as defined in any of claims 34-36 in an amount effective to modulate said DPP-I levels in said subject.

35

42. The method according to claim 41, wherein said DPPI is inhibited.

43. The method according to claim 42, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Cathepsin B})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
- 5 44. The method according to claim 42 or 43, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Cathepsin G})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
- 10 45. The method according to any of claim 42-44, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Cathepsin H})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
- 15 46. The method according to any of claim 42-45, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Cathepsin L})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
- 20 47. The method according to any of claim 42-46, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{DPP-IV})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
48. The method according to any of claim 42-47, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Neutrophil elastase})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.
- 25 49. The method according to any of claim 42-48, wherein DPP-I is selectively inhibited as determined by  $IC_{50}(\text{Tryptase})/IC_{50}(\text{DPP-I assay})$  is 25 or more such as, e.g., 50 or more, 75 or more or 100 or more.

From the INTERNATIONAL SEARCHING AUTHORITY

**PCT**NOTIFICATION OF DECISION CONCERNING  
REQUEST FOR RECTIFICATION

(PCT Rule 91.1(f))

To:

ALBIHNS A/S.  
Attn. Marianne Johansen  
H.C. Andersens Boulevard 49  
DK-1553 Copenhagen V  
DENMARKDate of mailing  
(day/month/year) 23/08/2004

Applicant's or agent's file reference

P10912 PC

REPLY DUE

NONE  
However, see last paragraph below

International application N°.

PCT/DK2004/000371

International filing date  
(day/month/year)

27/05/2004

Applicant

COMBIO A/S

The applicant is hereby notified that this International Searching Authority has considered the request for rectification of obvious errors in the international application/in other papers submitted by the applicant to this Authority, and that it has decided:

1. ☐ to authorize the rectification:☐ as requested by the applicant.☐ to the extent set forth below\*:2. ☒ to refuse to authorize the rectification or part of it for the following reasons\*:

The rectification of the error (i.e. the introduction of the feature "at 20  $\mu$ M concentration") is not obvious in the sense that anyone would immediately realize that nothing else could have been intended.

A copy of this notification, together with a copy of the applicant's request for rectification, has been sent to the receiving Office and to the International Bureau.

\* If the authorization of the rectification has been refused in whole or in part, the applicant may request the International Bureau, before the technical preparations for international publication have been completed and subject to the payment of a fee, to publish the request for rectification together with the international application. See Rule 91.1 (f), third and fourth sentences, and, for the amount of the fee, see the PCT Applicant's Guide, Volume I/A, Annex B2(1B).

Name and mailing address of the International Searching Authority



European Patent Office, P.B. 5818 Patentlaan 2  
NL-2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Marthe Oldendorf

(Schafer-N, Denmark) as a substrate. For each measurement, cell lysate corresponding to approximately 50 µg of protein was used. All samples were prepared in duplicates.

5 Assay for determination of metabolic stability is rat liver microsomes

The assay was performed at Cerep (Seattle) according to the protocol described by Kuhn et. al. (Kuhn, W., Gieschen, H. *Drug Metab. Dispos.*, 26, 1120-1127), using rat liver microsomes (0.3 mg/mL), test compound (1 µM), NADP (1 mM), and G6P (5 mM), and G6PDHase (1 U/mL) with 0.6 % methanol/0.6 % acetonitrile in water. Incubation was performed at 37 °C in phosphate buffer (50 mM, pH = 7.4), and the samples were analyzed for remaining product ion (selected reaction monitoring) by HPLC-MS/MS (Thermo Finnigan) at 0, 15, 30, 45 and 60 min. intervals.

Results:

15

Assay	Enzyme/ species	Example 11
Primary enzymatic screening (IC <sub>50</sub> )	DPP-I	40 ± 6 nM
Secondary enzymatic screening (10 µM cutoff or IC <sub>50</sub> )	Cathepsin B	4500 nM
	Cathepsin G	> 10 µM
	Cathepsin H	3200 nM
	Cathepsin L	> 10 µM
	DPP-IV	> 10 µM
	Neutrophil elastase	> 10 µM
Metabolism: CYP enzymes (20 µM cutoff or IC <sub>50</sub> )	Tryptase	> 10 µM
	CYP1A2	> 20 µM
	CYP2C9	> 20 µM
	CYP2C19	> 20 µM
	CYP2D6	> 20 µM
Cellular (% inhibition at 20 µM concentration)	CYP3A4	600 nM
	DPP-I (RBL-2H3 cells)	54%
Metabolic stability (t <sub>1/2</sub> in min.)	Rat liver microsomes	23

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/DK2004/000371

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C281/06 C07D295/14 C07D307/52 C07D209/16 C07D317/58  
C07D333/20 C07D211/56 C07D207/08 C07D207/26 C07K5/06  
A61K31/175 A61P29/00 A61K31/38 A61K31/40 A61K31/435

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/04277 A (UNIV PITTSBURGH) 5 February 1998 (1998-02-05)  figure 4; compound 19	1-3,7, 10-13, 29-39
X	FROCHOT C ET AL: "A SOLID-PHASE SYNTHESIS OF THREE AZA-, IMINOAZA- AND REDUCED AZA-PEPTIDES FROM THE SAME PRECURSOR" LETTERS IN PEPTIDE SCIENCE, ESCOM SCIENCE PUBLISHERS, NL, vol. 4, no. 4-6, 1997, pages 219-225, XP001037193 ISSN: 0929-5666 page 221; figure 1; compound 2  ----- -/-	1,2,7, 11,12, 14,16, 19,22, 23,25, 29-39

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 July 2004

Date of mailing of the international search report

06/08/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Fitz, W



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK2004/000371

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HYUNSOO H ET AL: "Investigations of azapeptides as mimetics of Leu-enkephalin" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 8, no. 1-6, 6 January 1998 (1998-01-06), pages 117-120, XP004136634 ISSN: 0960-894X page 117; figure 1; compound 4	1,2,7, 10-12, 14,16, 19,22, 29-39
X	EP 0 083 849 A (ICI PLC) 20 July 1983 (1983-07-20)  page 15, line 21; compound C.	1,2,7, 10-14, 16,19, 20,22, 23,25, 29-39
X	EP 0 076 557 A (ICI PLC) 13 April 1983 (1983-04-13)  page 52, line 18	1,2,7, 10-14, 16,19, 20,22,29
X	US 4 407 794 A (ROQUES BERNARD ET AL) 4 October 1983 (1983-10-04)  column 1: formula (I) wherein A is AzaGly column 6 - column 7; table 1; compounds 6,8,18,19	1,2,7, 10-14, 16,19, 20,22, 23,25, 29-39
X	DE 27 15 803 A (ICI LTD) 27 October 1977 (1977-10-27)  table 1; compound 10	1,2,7, 10-12, 14,16, 19,22, 23,25, 29-39
X	GRAY, C. J. ET AL: "Synthesis of azaalanine peptides using the solid phase method" BIOMEDICAL PEPTIDES, PROTEINS & NUCLEIC ACIDS , 2(1), 13-18 CODEN: BPPAFS; ISSN: 1353-8616, 1996, XP008033349 page 17; compound GM(D)	1,2,4,7, 10-12, 14,16, 19,22, 29-39
	-/--	

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK2004/000371

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAYNTER, H. ET AL: "Synthesis of deltorphin I analogs and preliminary characterization of $\mu$ -receptor binding" BIOCHEMICAL SOCIETY TRANSACTIONS , 21(3), 262S CODEN: BCSTB5; ISSN: 0300-5127, 1993, XP008033355 table 1; compound (AZAALA)2DELTORPHIN	1,2,7, 10-12, 14,16, 19,20, 22,29-39
X	DUTTA, ANAND S. ET AL: "Polypeptides. Part 15. Synthesis and biological activity of $\alpha$ -aza analogs of luliberin modified in positions 6 and 10" JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1: ORGANIC AND BIO-ORGANIC CHEMISTRY (1972-1999) , (2), 379-88 CODEN: JCPRB4; ISSN: 0300-922X, 1979, XP001194638 page 381, column 2, line 4; compounds 30A-D	1,2,7, 10-14, 16,19, 22,29-39
X	NIEDRICH, HARTMUT ET AL: "Hydrazine compounds as hetero-components in peptides. XIX. Eledoisin peptides containing the N2-methylcarbazoyle radical (azaalanine)" JOURNAL FUER PRAKTISCHE CHEMIE (LEIPZIG) , 316(5), 741-9 CODEN: JPCEAO; ISSN: 0021-8383, 1974, XP008033357 page 742; compound 2	1,2,4,7, 10-12, 14,16, 19,20, 22,29-39
X	NIEDRICH, HARTMUT ET AL: "Hydrazine compounds as hetero-components in peptides. XV. Synthesis of eledoisin octapeptides with the carbazyl residues azaglycine and $\alpha$ -azaasparagine instead of glycine and asparagine" JOURNAL FUER PRAKTISCHE CHEMIE (LEIPZIG) , 314(5-6), 759-68 CODEN: JPCEAO; ISSN: 0021-8383, 1972, XP008033358 page 761; compound 8	1,2,4,7, 10-14, 16,19,22
A	GB 992 961 A (CIBA LTD) 26 May 1965 (1965-05-26) examples 10,11	1,30-34, 37,39-41
A	NEUMANN, U. ET AL: "Reaction of dipeptidylpeptidase IV with substrate-analogous azapeptides" JOURNAL OF ENZYME INHIBITION , 4(3), 213-26 CODEN: ENINEG; ISSN: 8755-5093, 1991, XP008033354 page 217, line 1	1,30-34, 37,39-41
	-/-	

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/DK2004/000371

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/20804 A (OLSEN JOHAN GOTTHARDT ;LARSEN SINE (DK); PEDERSEN JOHN (DK); PROZY) 14 March 2002 (2002-03-14) the whole document	1,5-8
A	US 5 602 102 A (MCGUIRE MICHAEL J ET AL) 11 February 1997 (1997-02-11) cited in the application the whole document	1,5-8
A	WO 03/022871 A (HEISER ULRICH ;PROBIODRUG AG (DE); NIESTROJ ANDRE (DE); DEMUTH HAN) 20 March 2003 (2003-03-20) cited in the application the whole document	1,5-8
A	WO 99/59570 A (SMITHKLINE BEECHAM CORP ;HALBERT STACIE MARIE (US); THOMPSON SCOTT) 25 November 1999 (1999-11-25) the whole document, in particular: page 33, line 1; page 37, line 24; example 15 and page 65, line 13; page 56, line 18; claims 6 and 20	1,30-34, 37,39-41
A	WO 97/16433 A (DESJARLAIS RENEE LOUISE ;GALLAGHER TIMOTHY FRANCIS (US); SMITHKLIN) 9 May 1997 (1997-05-09) the whole document, in particular: page 244, line 13 and lines 14-15; claims 63 and 72	1,30-34, 37,39-41
A	DATABASE CHEMCATS Chemical Abstracts Service, Columbus, Ohio, US; XP002263984 Order number: BAS 0067804 & "INTERCHIM INTERMEDIATES" 9 July 2002 (2002-07-09), INTERCHIM , MONTLUCON, CEDEX, 03103, FRANCE	1,30-34, 37,39-41
A	EP 0 496 393 A (HOECHST AG) 29 July 1992 (1992-07-29) claim 1; examples	1,30-34, 37,39-41
A	TIEN, N. B. ET AL: "Tuberculostatic N-arylglycines and derivatives" JOURNAL OF ORGANIC CHEMISTRY, 23, 186-8, 1958, XP002263983 page 187, Formula III;	1,30-34, 37,39-41
	-/--	

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/DK2004/000371

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MISRA, VINAY S. ET AL: "Possible antituberculous compounds. XV. N-2-Thiazolyglycines, 1-acyl-4-arylsemicarbazides, 1-acyl-4-arylthiosemicarbazides, and N1-(N-arylglycyl)-N2-(arylidane or alkylidene) hydrazines" J. INDIAN CHEM. SOC., 40(9), 799-802, 1963, XP008025483 page 799, scheme (2): A and reaction product of A with alpha-naphtyl isocyanate; page 801, Table III	1,30-34, 37,39-41

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK2004/000371

### Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: —  
because they relate to subject matter not required to be searched by this Authority, namely:  

Although claims 37,38 and 41-49 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK2004/000371

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9804277	A	05-02-1998	US 5952294 A	14-09-1999
			AU 735243 B2	05-07-2001
			AU 3652797 A	20-02-1998
			CA 2261886 A1	05-02-1998
			EP 0918534 A1	02-06-1999
			JP 2002508736 T	19-03-2002
			WO 9804277 A1	05-02-1998
EP 0083849	A	20-07-1983	DE 3265905 D1	03-10-1985
			EP 0083849 A1	20-07-1983
			JP 58174350 A	13-10-1983
			US 4476117 A	09-10-1984
EP 0076557	A	13-04-1983	AT 16495 T	15-11-1985
			DE 3267380 D1	19-12-1985
			EP 0076557 A2	13-04-1983
			JP 57212148 A	27-12-1982
			US 4421744 A	20-12-1983
US 4407794	A	04-10-1983	FR 2488253 A1	12-02-1982
			AT 10836 T	15-01-1985
			CA 1292344 C	19-11-1991
			DE 3167826 D1	31-01-1985
			EP 0046113 A1	17-02-1982
			JP 57059845 A	10-04-1982
DE 2715803	A	27-10-1977	GB 1523812 A	06-09-1978
			AU 510760 B2	10-07-1980
			AU 2381977 A	05-10-1978
			BE 853448 A1	10-10-1977
			CS 202568 B2	30-01-1981
			DD 130348 A5	22-03-1978
			DE 2715803 A1	27-10-1977
			DK 158077 A	09-10-1977
			FI 771049 A	09-10-1977
			FR 2347336 A1	04-11-1977
			HU 176260 B	28-01-1981
			IE 44281 B1	23-09-1981
			JP 52139039 A	19-11-1977
			NL 7703851 A	11-10-1977
			NO 771237 A	11-10-1977
			NZ 183712 A	25-10-1979
			PL 197302 A1	13-02-1978
			SE 7704043 A	09-10-1977
			SU 904518 A3	07-02-1982
			ZA 7701789 A	29-03-1978
GB 992961	A	26-05-1965	GB 992962 A	26-05-1965
			US 3250760 A	10-05-1966
WO 0220804	A	14-03-2002	AU 3815102 A	22-03-2002
			CA 2422057 A1	14-03-2002
			WO 0220804 A1	14-03-2002
			EP 1317555 A1	11-06-2003
			US 2004043469 A1	04-03-2004
US 5602102	A	11-02-1997	AU 4396993 A	30-12-1993
			DE 4392555 T0	27-04-1995

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK2004/000371

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5602102	A		WO 9324634 A1	09-12-1993
WO 03022871	A	20-03-2003	DE 10143840 A1	27-03-2003
			WO 03022871 A2	20-03-2003
			EP 1423410 A2	02-06-2004
			US 2003119738 A1	26-06-2003
WO 9959570	A	25-11-1999	AU 9110298 A	06-12-1999
			CA 2332492 A1	25-11-1999
			EP 1079821 A1	07-03-2001
			JP 2002515428 T	28-05-2002
			WO 9959570 A1	25-11-1999
WO 9716433	A	09-05-1997	AU 1118097 A	22-05-1997
			AU 711014 B2	07-10-1999
			AU 1270797 A	17-07-1997
			BG 101712 A	27-02-1998
			BG 102475 A	30-04-1999
			BR 9607577 A	14-09-1999
			BR 9612344 A	13-07-1999
			CA 2209109 A1	09-05-1997
			CA 2236111 A1	09-05-1997
			CN 1341590 A	27-03-2002
			CN 1341592 A	27-03-2002
			CN 1341593 A	27-03-2002
			CN 1177293 A	25-03-1998
			CZ 9801327 A3	17-02-1999
			CZ 9702060 A3	17-02-1999
			EP 0934291 A1	11-08-1999
			EP 0804180 A1	05-11-1997
			HU 9802488 A2	01-02-1999
			HU 9900964 A2	28-07-1999
			JP 10512300 T	24-11-1998
			JP 2000500742 T	25-01-2000
			NO 973009 A	27-08-1997
			NO 981938 A	29-06-1998
			NO 20006716 A	29-06-1998
			NO 20006717 A	29-06-1998
			NO 20006718 A	29-06-1998
			NZ 324101 A	29-11-1999
			NZ 324921 A	28-07-2000
			OA 10786 A	24-12-2002
			OA 10764 A	11-12-2002
			PL 326508 A1	28-09-1998
			PL 328877 A1	01-03-1999
			SK 48198 A3	07-10-1998
			SK 56798 A3	02-12-1998
			SK 88997 A3	06-05-1998
			TR 9700560 T1	21-11-1997
			TR 9800767 T2	21-07-1998
			WO 9716177 A1	09-05-1997
			WO 9716433 A1	09-05-1997
			US 2002173469 A1	21-11-2002
			US 5998470 A	07-12-1999
			US 6057362 A	02-05-2000
			US 6232342 B1	15-05-2001
			US 6331542 B1	18-12-2001
			US 6284777 B1	04-09-2001

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK2004/000371

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9716433	A	US 2002077455 A1	20-06-2002
EP 0496393	A	29-07-1992	
		AT 117673 T	15-02-1995
		CA 2059909 A1	25-07-1992
		DE 59201241 D1	09-03-1995
		DK 496393 T3	26-06-1995
		EP 0496393 A1	29-07-1992
		ES 2069322 T3	01-05-1995
		FI 920270 A	25-07-1992
		GR 3015103 T3	31-05-1995
		IE 920201 A1	29-07-1992
		JP 3018264 B2	13-03-2000
		JP 4334360 A	20-11-1992
		MX 9200289 A1	01-07-1992
		NO 920312 A ,B,	27-07-1992
		US 5326875 A	05-07-1994